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AMIDOPYRINE HYPERSENSITIVITY*

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IN RECENT years the literature has been replete with reports of amidopyrine toxicity. The most frequently reported toxicity has been granulocytopenia. In 1918, Haythorn,[1] in 1926 Willcox,[2] Rabinowitz in 1930,[3] and in 1932 Weis[4] called attention to the fact that the benzene nucleus itself might be a cause of drug intoxication. In 1933, Beck[5] hinted that granulopenia may be due to drugs, particularly coal tar drugs, containing the benzene ring. She referred to previous purely experimental work in rabbits by Kracke who had apparently produced granulopenia with no apparent effect on either erythrocytes or platelets. Early in 1933, Madison[6] alluded to the subject and later in the year Watkins published on the relation of barbiturates and amidopyrine in the causation of leukopenia.[7] The consensus of opinion since that publication, however, has cast great doubt upon the causation of granulocytopenia with any appreciable frequency by barbiturates.[8] Late in 1933, Madison and Squier,[9] and again early in 1934,[10] published on the etiology of primary granulocytopenia and implicated the benzene nucleus. Early in 1934, Thompson[11] published on the possible relation between female sex hormone and agranulocytosis. About this time, Holten and associates[12] called attention in Copenhagen to this relation between granulocytosis and patients on amidopyrine. In April of the same year, Randall[13] published on the same subject, also believing that barbiturates sometimes cause granulopenia; and Hoffman, Butt and Hickey[14] published in a similar vein on amidopyrine. In May, 1934, Hardwick and Randall, of the Mayo Clinic, published their findings on the leucocyte content of the blood of patients following obstetric analgesia with pentobarbital sodium and found no granulocytopenia cases.[15] In the following month, Rawls[16] published

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on the development of neutropenia following amidopyrine medication, and likewise Zinberg and associates.[17] In August, 1934, Fitz-Hugh reviewed this subject.[18] Johnson[19] and Herz[20] published along similar lines in October. At about this time, Roy Kracke[21-24] vigorously called attention to the fact that he believed granulocytopenic intoxication to be due to the presence of the benzene ring. In 1935, the following authors published upon this general subject: Jackson and Parker;[25, 26] Fishbein;[27] Editor of the *British Medical Journal*;[28] Boulton;[29] Bolton;[30] Russell Haden;[31] Hansen and Holten;[32] Hewlett;[33] Hunter;[34] Limarzi and Murphy;[35] Plum;[36] Saint-Germain;[37] and Stenn.[38]

In summary, it may be said that there are three theories as to the etiology of amidopyrine intoxication resulting in granulocytopenia; namely: (a) the theory of Kracke and associates that all drugs containing the benzene nucleus, or the benzene nucleus in association with an amino group, are per se toxic, presumably to the bone marrow; (b) the theory of Climenko[39] that amidopyrine is semispecific for the bone marrow and produces a direct intoxication thereof; (c) the allergic theory of Dameshek and associates.[40] Some comment upon these theories seems in order at this point.

First, the theory proposed by Kracke[22] that the disease appears to be more prevalent among physicians, their relatives, nurses, etc., is a very questionable statistical conclusion. It is true that cases are more frequently reported in that group than in any other group. He bases this conclusion on the following:

1. Physicians are prone to prescribe and use this kind of drug in their own families.

2. Hospital nurses in the course of duty, when seeking a mild analgesic, because of their familiarity with these lesser known preparations, will employ one of them instead of aspirin.

3. The average layman, when treating his own aches and pains, will use aspirin.

However, it must be remembered that the lay use of mixtures and patent medicines, containing amidopyrine and drugs of that nature, is enormous. It is much more likely that the medical and nursing professions are more sensitively introspective, and in general receive much more careful medical care in diagnosis than the general lay population; hence, the greater number of reports in this class.

The statement that the benzene nucleus per se, or the benzene nucleus in association with a substituted amino group, is causative of agranulocytosis, seems unlikely. Points against this reasoning are: (1) the benzene nucleus is the foundation stone for all aromatic compounds, hence, one must assume that all aromatic compounds are similarly toxic, which supposition we know to be false; (2) if the benzene nucleus in association with an amino group is toxic, is it not curious that two of the most fundamental hormones in the human body, namely thyroxin and epinephrine, both contain the benzene nucleus in association with, although not attached to, an amino group? Incidentally, in that essential amino acid, tryptophane, the substituted amino group is attached directly to the benzene nucleus. (3) The term "benzamine" can properly be applied only to aniline, its application otherwise being a misnomer. Miscel-

laneous compounds containing nitrogen are not necessarily amines. From the discussion of some of the writers in the literature, one would expect them to classify nitric acid as an amine! It seems unusual that if the benzene nucleus is toxic the following normal constituents of the body or the diet all contain the benzene nucleus. These are, phenylalanine, hippuric acid, parahydroxybenzoic acid, tyrosine, tryptophane, parahydroxyphenylpropionic acid, indoxyl, sulphuric acid, parahydroxyphenylethylamine, etc., etc. One might go further and name other compounds but it is scarcely necessary. (4) If the benzene nucleus per se is so toxic, is it not curious that with such enormous quantities of sodium salicylate as have been given in the last thirty or forty years, that granulopenia has not been noted as a prominent result of that medication? (5) The attempt by Kracke and his associates to differentiate so strongly between barbiturate and amidopyrine is perfectly praiseworthy but not upon the ground that they differ, i.e., the one is the benzene derivative and the other a straight chain compound. As everyone knows, the most commonly used barbiturate, namely, phenobarbital, does contain the benzene nucleus, and Shonle[41] has published voluminously upon barbiturates containing ring structures.

Second, the theory of Climenko does not go very far to explain why some people get agranulocytosis and others do not. Furthermore, Limarzi and Murphy,[35] and others, have reported cases where amidopyrine was administered right straight through the attack and convalescence from an attack of agranulocytosis without deleterious effects. The author believes, however, that this explanation of Climenko,[39] if coupled with the explanation of Thompson[11] as to female sex hormone, might adequately explain the phenomenon as far as females are concerned.

Third, the very ingenious method of Dameshek employed to demonstrate the allergic nature of amidopyrine intoxication, dovetailing so accurately as it does with the allergic theory of Armand J. Quick[42] on the cause of cinchophen intoxication comes close to being a satisfactory explanation of the whole situation. No one theory, however, can explain all the phenomena. Reznikoff,[43] stresses the point that fatigue, infection, age, and menstruation play an important part in the production of the disease.

In an editorial in the *J. A. M. A.*[8] in a special report of the Council on Pharmacy and Chemistry, the case against amidopyrine is summed up as follows: "When one considers the enormous amount of amidopyrine consumed and the relatively few individuals afflicted with granulocytopenia, it is obvious that one is dealing with a question of sensitivity in certain patients rather than with a universal action of the drug."

Since Schultz[44] published his article in 1931 on the use of amidopyrine in rheumatic fever, we have been using this drug extensively in the arthritic clinics of St. Luke's Hospital and the New York Post-Graduate Hospital and also in private practice. Schultz concluded his article by saying that "instances of idiosyncrasy are less frequent than with many other drugs of this type—the feasibility of giving effective quantities, without provoking untoward side actions as well as the wide margin between the therapeutic and toxic doses, renders this drug particularly valuable in the treatment of patients with rheumatic fever."

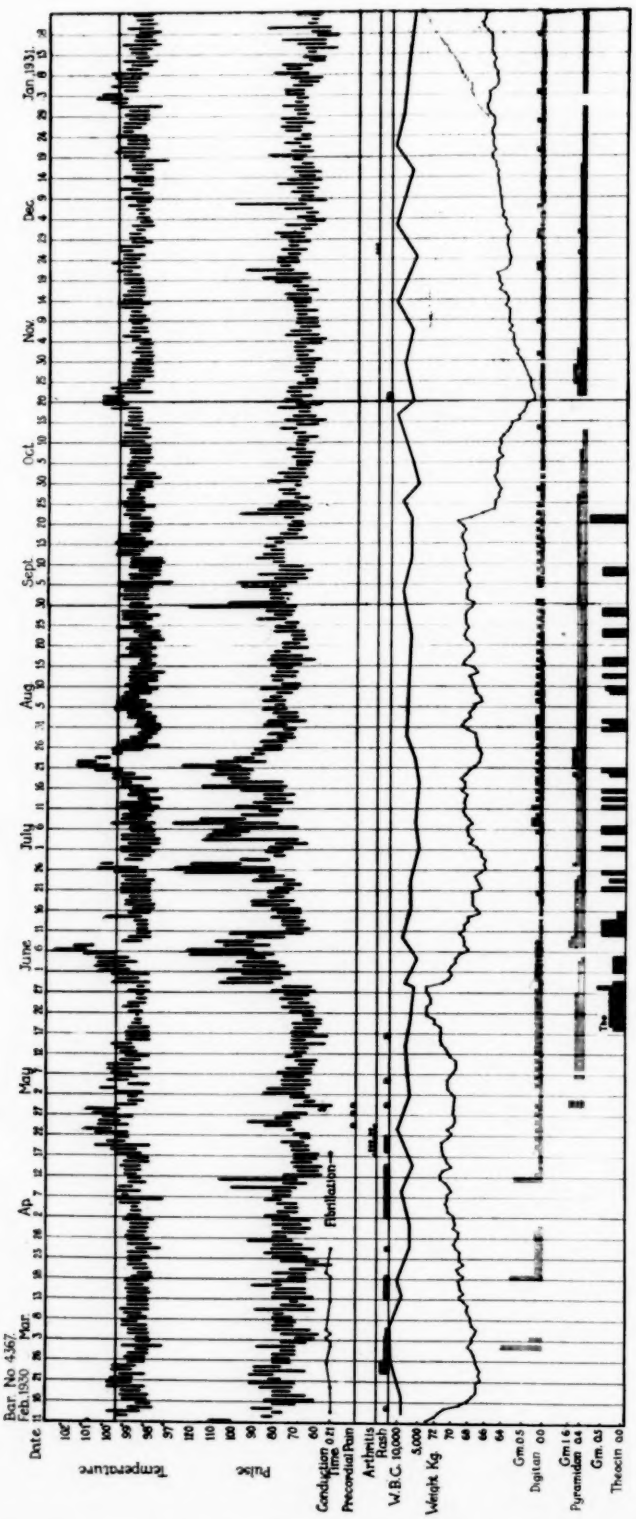


Chart 1.—Effect of amidopyrine on leucocyte count of patient followed by Schultz at the Rockefeller Hospital.

Muller,[15] at one time gave as much as 10 gm. in a day and continued the administration at a level from 4 to 6 gm. daily, without any ill effects. We have reviewed our records for the past two years in order to ascertain whether those taking amidopyrine had undergone a reduction in the granulocytes of their blood. Unfortunately, very few of these had leucocyte counts on more than one occasion for there seemed to be no indication for such determinations. None of these patients complained of any severe ill effects, although an occa-

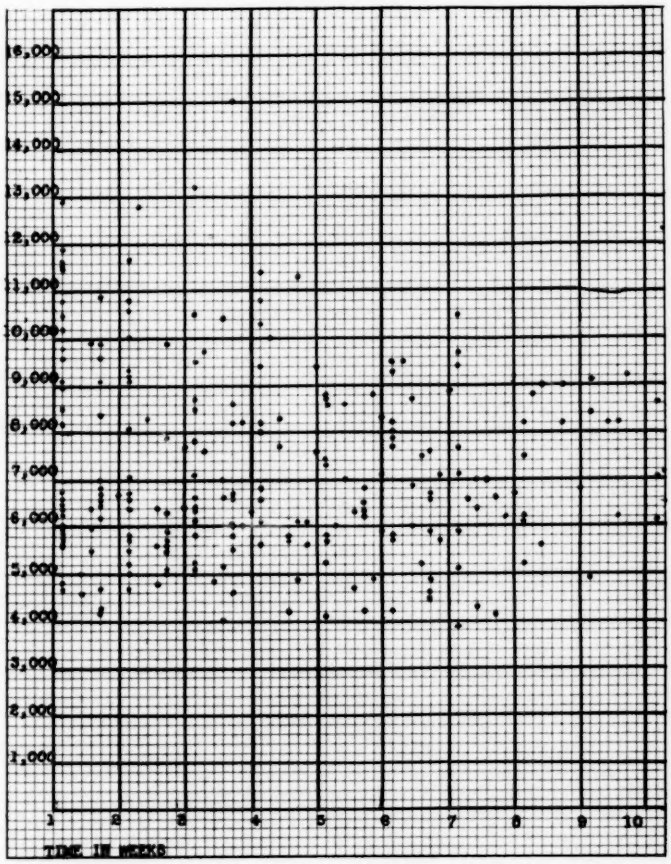


Chart 2.—Frequency chart of total white count of 32 patients.

sional one complained of loss of appetite or dizziness, and the drug was discontinued for a few days. These results are summarized in Table I.

Dr. Mark B. Schultz has very kindly allowed me to review the charts of the patients at the Hospital of the Rockefeller Institute, from which he collected his data for his paper on the use of amidopyrine in rheumatic fever. These records are well kept and frequent leucocyte counts were done. Chart 1 is a typical one taken from Schultz's paper. It can be seen that there was no particular fluctuation in the leucocyte count. He studied 32 cases and in no instance was there a drop in the total white count.

TABLE I

EFFECT OF AMIDOPYRINE ON LEUCOCYTE COUNT OF SIXTEEN PATIENTS FOLLOWED OVER A PERIOD OF SEVERAL YEARS IN THE CLINIC OF ST. LUKE'S HOSPITAL

NAME	BLOOD COUNT BEFORE	DATE	AMIDOPYRINE, FIRST	DATE	AMIDOPYRINE, LAST	DATE	BLOOD COUNT AFTER	DATE
H. A.	6,700 (58-42)	2/24/33	Gr. V, p. c.	--------	Gr. V, b. d., t. i. w.	--------	5,200	11/ 9/34
M. B.	6,800 (72-28)	3/ 3/34	Gr. X, p. c.	5/25/34	Gr. X, p. c.	10/ 5/34	10,400 (54-46)	3/ 1/35
E. F.	11,100	11/10/34	Gr. X, p. c.	11/10/34	----------	--------	7,000 (52-48)	3/ 1/35
M. F.	9,400 (50-54)	7/20/32	Gr. V, p. c.	8/11/33	Gr. X, p. c.	6/ 6/34	9,200	4/ 6/34
J. H.	9,100	5/ 4/34	Gr. X, p. c.	5/ 4/34	Gr. X, p. c.	11/16/34	9,200	7/ 3/34
C. H.	----	--------	Gr. V, t. i. d.	6/12/34	Gr. X, p. c.	11/16/34	8,200 (60-40)	11/30/34
L. I.	7,200	--------	Gr. X, p. c.	5/ 4/34	Gr. X, p. c.	10/ 5/34	6,800 (68-32)	11/30/34
A. L.	10,300	4/22/32	Gr. V, p. c.	7/ 8/32	Gr. X, p. c.	10/12/34	6,400 (66-34)	11/30/34
L. L.	7,400 (68-32)	9/27/33	Gr. X, p. c.	4/13/34	Gr. X, p. c.	5/25/34	6,900 (60-39)	11/30/34
H. M.	----	--------	Gr. V, t. i. d.	7/14/32	Gr. X, p. c.	6/22/34	13,200 (72-28)	12/14/34
V. M.	----	--------	Gr. V, t. i. d.	5/ 5/33	Gr. V, t. i. d.	6/16/33	----------	--------
M. P.	8,400	10/17/34	Gr. X, p. c.	10/19/34	----------	--------	8,000 (65-35)	1/11/35
A. P.	12,200	4/ 6/34	Gr. X, p. c.	4/20/34	Gr. X, p. c.	6/ 8/34	9,100	7/ 3/34
H. S.	7,900 (80-20)	8/24/34	Gr. X, p. c.	9/14/34	----------	--------	6,000 (68-30)	11/23/34
E. S.	10,200	5/ 4/34	Cont. 8/12/32	--------	Cont. 6/8/34	--------	7,700	6/ 8/34
I. W.	9,850	5/11/34	Gr. V, p. c.	5/11/34	Gr. V, p. c.	6/29/34	8,660	10/ 4/34

Thirty-two patients have been selected and given 30 gr. of amidopyrine (pyramidon) daily, and an attempt made to do total leucocyte and differential counts twice weekly. In some instances the patients did not return but many were faithful and came back regularly. Twenty-three of the cases were followed at St. Luke's Hospital by a single technician and the counts done at the same time of day each week. Nine of the patients were studied at New York Post-Graduate Hospital by another technician who followed the same routine as was followed at St. Luke's. Two hundred cells were counted in doing the differentials. The 32 patients studied came to the clinic because of arthritis, either of the rheumatoid or osteoid type.

The patient in Case 13 had, in addition to rheumatoid arthritis, pernicious anemia, and the patient in Case 21 had, in addition to rheumatoid arthritis, a sensitivity to aspirin, a polyglandular dyscrasia, and, on several occasions, myelocytes were found in her blood. Her mother and father were first cousins and one brother was insane, while another brother had some congenital defect. These two were chosen because it was thought that their marrow would be particularly susceptible to any toxic agent, if such were present in amidopyrine. The frequency curve given in Chart 2 is made from counts of the 32 patients. In no instance was there a significant drop in the white cell count. The blood findings in these 32 cases are summarized in Table II.

Intracutaneous skin tests were done with 5 per cent solution of amidopyrine on 50 patients, 2 of whom were normal; one had had two severe attacks of agranulocytosis, and 47 had arthritis. These were selected in the main from the 32 reported above. Saline solution containing 100,000 dead hemolytic streptococci per c.c. was used as a control in most instances—0.05 c.c. of each was used in making the test. In only one case could the reaction be called positive. In each there was some redness at the site of the injection of the amidopyrine for a few minutes after the solution was given. As this was invariable, it was interpreted as being of no consequence. In the instance where the positive reaction was obtained, the patient was extremely sensitive to amidopyrine by mouth. Five grains would make her quite dizzy and nauseated.

In addition to the cases reported in this paper, the histories of the patients admitted to St. Luke's Hospital with a diagnosis of agranulocytosis have been studied. Also, the cases of polycythemia that received phenylhydrazine have been reviewed, in the belief that this substance might possibly affect not only the red cells but also the white cells. Only 2 cases of polycythemia, who had received phenylhydrazine, could be found, and, while the red blood cells were somewhat reduced, the white count was increased. These results are given in Table III. The benzene ring as it occurs in phenylhydrazine evidently stimulated the bone marrow to some extent in these two cases, or the liver destruction may have been responsible for the rise.

Twenty cases of agranulocytosis are here reported. Of these, 9 died and 11 recovered. Four of these cases had a history of receiving amidopyrine before entering the hospital. Three of these 4 recovered.

One patient, L. M., was admitted to the ward on September 15, 1929, with a diagnosis of residual hemiplegia. Her blood count was as follows: Hemoglobin, 86 per cent; red blood cells, 4,600,000; leucocytes, 9,000; polymorphonuclears, 82 per cent; lymphocytes, 18 per cent.

TABLE II

FIRST AND LAST TOTAL WHITE COUNT AND PERCENTAGE POLYMORPHONUCLEAR LEUCOCYTES AND LYMPHOCYTES IN THIRTY-TWO CASES STUDIED

NO.	NAME	DATE	WHITE COUNT	POLY.	LYM.
1	E. S.	May 4	10,200	70	30
		June 8	7,700	61½	37
2	L. L.	April 20	4,750	56	43½
		June 5	4,350	51	47½
3	J. H.	May 4	9,100	84½	14½
		July 3	9,200	71½	24
4	K. D.	May 4	6,400	76½	21
		June 12	4,650	57½	41½
5	A. P.	April 20	6,400	60½	38½
		July 3	9,100	62	36
6	A. N.	May 4	9,650	65½	34½
		June 15	6,600	58	38
7	G. K.	April 27	11,550	83½	16½
		June 15	6,100	53½	46
8	K.	May 4	10,500	58½	41
		June 15	10,550	47	51
9	J. B.	April 20	11,950	70	29½
		May 4	9,750	70	30
10	A. F.	May 4	5,750	58½	40½
		June 8	8,300	66½	31½
11	M. M.	April 27	5,800	66	34
		June 26	6,200	62½	33
12	A. W.	May 4	6,850	62	38
		June 15	7,750	62	34
13	M. S.	April 20	11,550	65½	34
		July 3	5,300	49	45
14	S. B.	April 24	11,550	61	37½
		April 27	9,950	57½	41½
15	E. T.	May 11	8,500	64½	34
		May 18	10,000	63½	34½
16	I. W.	May 11	9,850	74	25
		July 3	5,500	56	41
17	L. K.	May 18	10,800	74½	25½
		June 29	9,450	70	30
18	M. F.	May 25	5,600	68½	31
		June 15	5,500	57	39½
19	T. R.	May 25	12,900	60½	38½
		June 12	6,600	67	33
20	B.	May 25	4,750	41½	57½
		June 15	3,950	50	49½
21	M. K.	June 1	5,850	59	41
		July 3	6,200	69½	30½
22	N. D.	June 1	7,500	51½	47½
		June 26	6,350	46½	46
23	E. O.	June 8	8,650	64	32
		June 29	7,100	59	40½
24	M. S.	June 15	8,000	51	44
		June 29	7,150	66½	33½
25	M. S.	April 6	6,300	54½	38
		May 11	8,800	75	18
26	K. M.	April 3	8,500	52½	21½
		May 8	9,300	61	34
27	L. S.	April 10	5,800	67	26
		May 11	6,300	54	33
28	J. O.	June 12	9,100	79	16
		June 26	10,550	80	17
29	A. D.	June 5	5,950	48	47
		June 8	6,000	37	57
30	M. P.	June 8	6,350	59	31
		June 26	5,850	56	33
31	I. D.	June 19	8,200	49	42
		June 21	4,600	53	45
32	E. I.	May 29	6,000	32	62
		June 26	7,350	36	61

TABLE III

EFFECT OF PHENYLHYDRAZINE ON WHITE COUNT OF TWO PATIENTS

NAME	W.B.C.	POLYS.	LYMPH.	DRUGS ADMINISTERED	RESULTS
J. B.	14,500 . . F			Phenylhydrazine	
	15,400 . . L			10 gr. 12 days	Improved
M. B.	8,600 . . F			58.5 gr. 13 days	Improved
	16,000 . . L				

On Oct. 7, 1929, she began to take amidopyrine, 20 gr. every day. On Oct. 19, 1929, her count was as follows: Leucocytes, 2,300; polymorphonuclears, 8 per cent; lymphocytes, 92 per cent. Oct. 20, 1929, leucocytes, 1,200; lymphocytes, 100 per cent. She died on Oct. 24, 1929. She had received 280 gr. of amidopyrine in fourteen days.

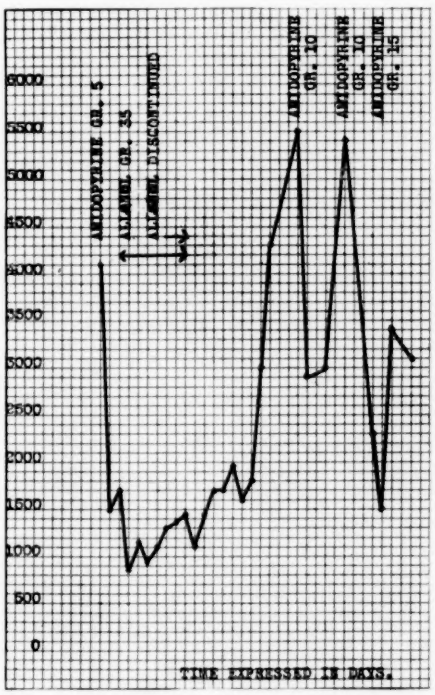


Chart 3.—The influence on the white count of patients taking amidopyrine and allanol.

Another patient, M. R., was admitted on April 8, 1928, with a diagnosis of chronic arthritis of the spine. Her count on that day was: White blood count, 9,000; polymorphonuclears, 59 per cent; lymphocytes, 39 per cent; eosinophiles, 2 per cent. On April 9, 1928, she was given: 5 gr. of aspirin, and 5 gr. of amidopyrine, four times a day. On April 21, 1928, she began to complain of a severe headache. April 29, 1928, white blood count, 525; polymorphonuclears, 10 per cent; lymphocytes, 90 per cent. She died on April 30, 1928 with a temperature of 105°. She had taken 200 gr. of amidopyrine in ten days.

C. K. was admitted on July 15, 1931, because of a sore throat. On July 19, 1931, his count was: White blood count, 3,200; polymorphonuclears, 56 per cent; lymphocytes, 44 per cent. On July 18, 1931, he had been given 10 gr. of amidopyrine. He had suffered from neuritis for several years and may have taken amidopyrine before, although it was not so stated in his history. He took 25 gr. of amidopyrine in three days, as well as 0.3 gm. of neosalvarsan. On July 31, 1931, his count was as follows: White blood count, 1,700; polymorphonuclears, 22 per cent; lymphocytes, 78 per cent. He died on Aug. 14, 1931.

S. B. was admitted on Dec. 12, 1928, complaining of headache, sore throat, and bleeding gums. His count was: White blood count, 7,500; polymorphonuclears, 76 per cent; lymphocytes, 22 per cent.

He had had some type of nose operation two months before. His tonsils had been removed at the age of seven. He had frequent colds and sore throats. On Dec. 17, 1928, he was started on 5 gr. of aspirin, 5 gr. of amidopyrine, 5 gr. of phenacetin, four times a day. On Dec. 18, 1928, his count was: White blood count, 1,200; polymorphonuclears, 6 per cent; lymphocytes, 90 per cent. On Dec. 20, 1928, his count was: White blood count, 1,100; polymorphonuclears, 2 per cent; lymphocytes, 94 per cent. He died on that date. He had taken about 200 gr. of amidopyrine.

A patient, J. P., received 40 gr. of amidopyrine, and 35 gr. of allylisopropylbarbituric acid with amidopyrine (allanol) during her acute illness. She recovered. On admission, on May 5, 1932, her count was: White blood count, 4,100; lymphocytes, 95 per cent. The next day her count had fallen to: White blood count, 1,500; and lymphocytes, 96 per cent. On the day of her admission, she had been given allanol, gr. v. By the thirteenth of the month she had been given 35 gr. of allanol and 5 gr. of amidopyrine. Her blood findings are summarized in Chart 3.

Another patient, B. F., who had taken some amidopyrine before admission to the hospital, recovered. The exact amount is not known. A skin test done a year after she left the hospital was negative. When given 5 gr. of amidopyrine, she became quite sick, felt nauseated, and became dizzy. Her count on the following day was: White blood count, 4,000; polymorphonuclears, 48 per cent; lymphocytes, 52 per cent. Her previous count, which was taken six months before, was: White blood count, 10,000; polymorphonuclears, 47 per cent; lymphocytes, 53 per cent.

F. B., a patient, was given 1.8 gm. of neosalvarsan. Blood counts were as follows:

W.B.C.	POLYS.	LYMPHOCYTES	NEOSALVARSAN	DATES
2,200	37%	63%	0.5	Sept. 24, 1930
2,800	40%	60%	--	Sept. 26, 1930
----	--	--	0.3	Oct. 2, 1930
----	--	--	0.5	Oct. 3, 1930
1,600	32%	68%	0.5	Oct. 4, 1930
1,400	20%	80%	--	Oct. 5, 1930
1,300	50%	50%	--	Oct. 6, 1930

He was given no more salvarsan and his count rose until on October 30, it was: White blood count, 5,600; polymorphonuclears, 70 per cent; lymphocytes, 30 per cent. This patient also had pernicious anemia. He recovered.

One patient died after taking antipyrine methyl amino methane sodium sulphate.* As this is the first case that we have been able to find, in which death was due to antipyrine methyl amino methane sodium sulphonate, the results are reported here in detail:

A white woman, a waitress, aged thirty-three years, was admitted to St. Luke's Hospital on Dec. 17, 1934. Her temperature was 105.4° F. Her leucocyte count was 1,100. This patient had been first seen on Feb. 20, 1934, and has been under observation since that time. She had first sought medical aid because of a low grade rheumatoid arthritis, involving her wrists and fingers, as well as her ankles. On the day that she was first observed, her hemoglobin was 87 per cent. Her red cell count was 4,575,000; white cell count 5,800. There were 61 per cent polymorphonuclears; 3 per cent eosinophiles; 32 per cent small lymphocytes; and 4 per cent large mononuclears. The urinalysis was negative. Her sedimentation rate was 5 mm. in one hour. The physical examination revealed nothing of importance save a fusiform swelling involving one proximal phalangeal joint, and moderately infected tonsils. She was given hemolytic streptococcus vaccine intravenously; the first dose was 50,000. She was given the

*Antipyrine methyl amino methane sodium sulphonate is the technical name for "Novaldin."

last dose of 1,300,000 on May 15. During this period, she had been taking 1 dr. of calcium gluconate, t.i.d., and 10 gr. of amidopyrine, t.i.d. She did not take amidopyrine regularly, so the exact dose is not known. She stated that she had taken this before she sought medical aid. During this period of observation, she frequently ran a temperature, rarely over 100°. It usually was higher in the middle of the day and returned to normal in the afternoon. Occasionally, she developed a nondescript erythematous rash on her legs. This, she said, she noticed particularly after she ate meat. On April 30, it was decided to give her 10 gr. of antipyrine methyl amino methane sodium sulphonate, t.i.d., instead of amidopyrine. Following this, her temperature disappeared and she felt perfectly well. At no time did she complain of indigestion. On May 15, she developed a pustular rash on her chest, which was slightly painful. This had the appearance of herpes zoster. This was painted with collodion, and she was given codeine for pain. On May 17, when she returned, the herpes had spread to the

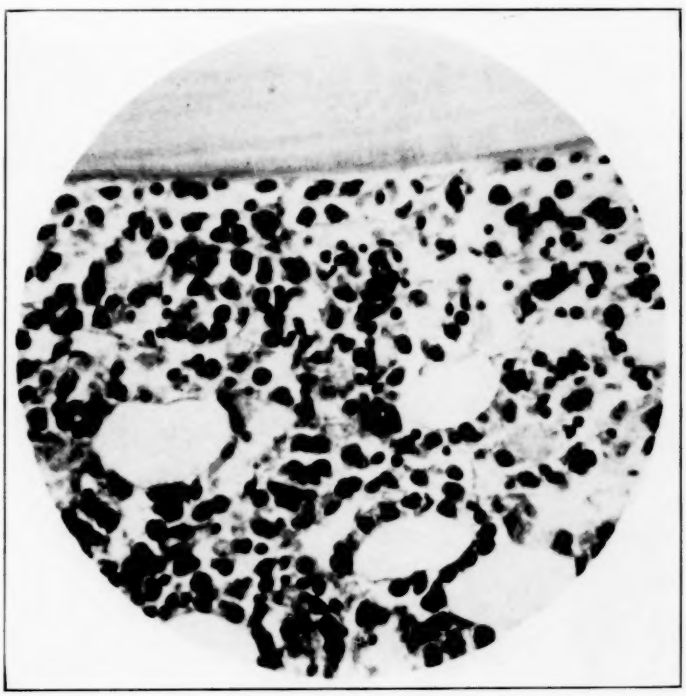


Fig. 1.—Low power photomicrograph of bone marrow of patient who died after taking novaldin.

other side of her chest and also to her lip. She was given an ampule of pituitrin and ten days later, was entirely well. On June 6, she came to the office complaining that she had, several days previously, had an attack of tonsillitis. At this time, she was advised to have her tonsils removed as soon as the attack subsided. On August 13, she was admitted to St. Luke's for a tonsillectomy, and the tonsils were removed without any ill effect. Following the removal of her tonsils, she felt quite well, except that her throat was red and sore. She was again complaining of some pain in her wrists and legs, when seen on November 5, and her throat was still red and sore. She was told to use an aspirin and soda gargle and to take 2 antipyrine methyl amino methane sodium sulphonate tablets after meals. On November 15, she developed a temperature and had muscular aches and pains and a very sore throat. She was seen on November 17, at which time, she had an ulcer in her posterior pharynx. Her temperature was 105°, and leucocyte count 1,100. She had taken approximately 72 tablets or 360 gr. of antipyrine methyl amino methane sodium sulphonate in twelve days. From this time on, she grew steadily worse, in spite of three transfusions of whole blood, injections of

pentneucleotide and liver extract. She died on November 21. The report made by the pathologist is as follows:

Report of Autopsy Findings (No. 3569).—"Pieces of bone and marrow are removed from the sternum and bodies of the second and third lumbar vertebrae, and marrow from the medullary cavity of the right femur. Aside from slight pallor, the marrow shows no gross pathologic changes."

Microscopic Examination.—"Bone from sternum and ribs. There is but moderate hyperplasia of the marrow elements which consists practically entirely of myelocytes, scattered myeloblasts and a fairly numerous number of plasma cells and lymphocytes. Normoblasts are present in fair proportion, but few megaloblasts are seen. There appears to be an increase in the number of megakaryocytes. No mature granulocytes are present; the arrest in maturation appearing to take place in the myelocytic phase. Small areas of necrosis are present which may be due to late fixation." These changes are shown in Figs. 1 and 2.

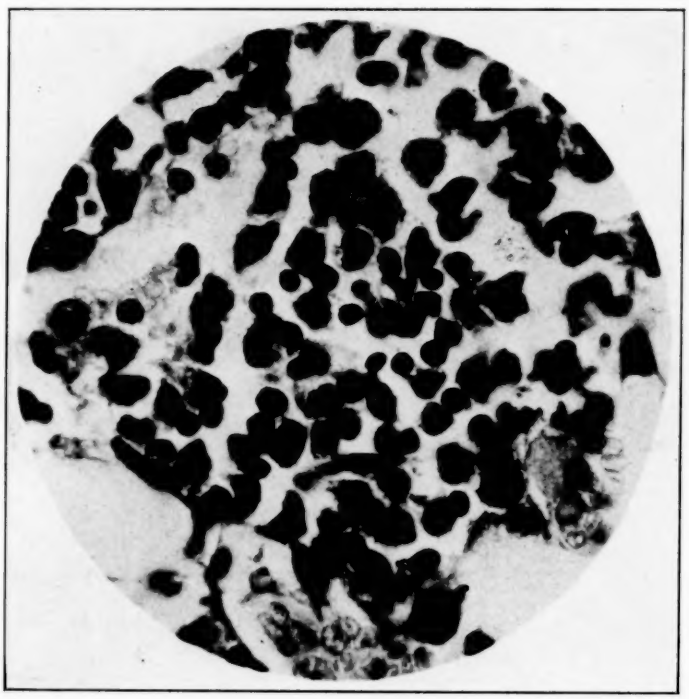


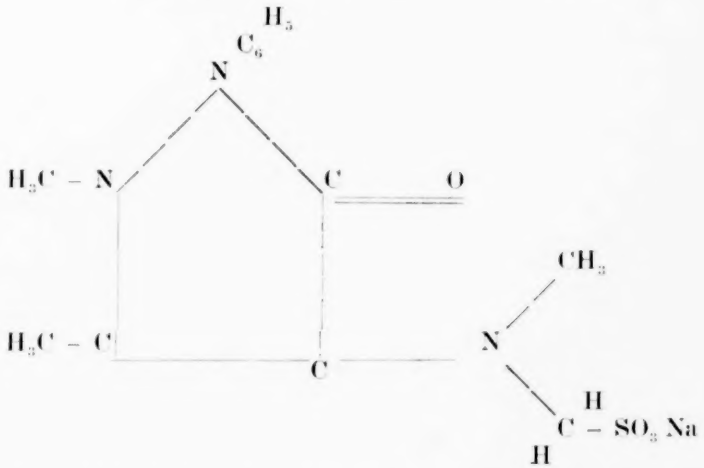
Fig. 2.—High power photomicrograph of bone marrow of patient who died after taking novaldin.

This case is of interest because it is the first one which has been known to be associated with antipyrine methyl amino methane sodium sulphonate. Moreover, this patient had large doses of amidopyrine and antipyrine methyl amino methane sodium sulphonate without any untoward symptoms until after her tonsils were removed. Following her tonsillectomy, her throat was red and sore until the day she died. She took antipyrine methyl amino methane sodium sulphonate for the second time, only during the last twelve days of her life. The autopsy findings were not those usually seen in a typical agranulocytosis.

In the typical agranulocytosis, when the patient dies within a few days of the onset, the bone marrow is made up entirely, so far as the white cell series is concerned, of stem cells. If the patient survives for a longer period, say ten days, these stem cells are gradually replaced by lymphocytes and plasma cells.

In the bone marrow of this case, there is a different situation. The white cells are represented for the most part by myelocytes, some in active mitosis. The red cells and megakaryocytes are normal. Thus, the maturation in this case has been arrested not at the stem cell stage, but at the myelocyte stage. It might be argued that the bone marrow was just beginning to recover and had reached the myelocyte stage when death occurred. When this course of events occurs, however, the marrow is grossly hyperplastic and this patient did not show such hyperplasia.

The structural form of antipyrine methyl amino methane sodium sulphonate is as follows:



Its toxicity for rabbits is $\frac{1}{40}$ of aspirin. It has been our clinical experience that many patients who are unable to take aspirin because of indigestion and other systemic disturbances, can tolerate antipyrine methyl amino methane sodium sulphonate. To date, we have found no one who is sensitive in any way to this drug, except the patient here reported. In a recent article in the *J. A. M. A.* Benjamin and Biederman[16] report a patient who was sensitive to novaldin.

DISCUSSION

Amidopyrine was first discovered and used by Filekne[47] in 1897. Agranulocytosis was discovered as a clinical entity thirty-four years ago or six years after the drug which is thought to be responsible for a large number of the cases. However, it was not until fifteen years ago that it attracted any particular attention. This discrepancy might be explained on the grounds that only in recent years the drug has received wide popularity and that the disease occurred before but was not recognized. The latter, however, seems unlikely because of the ease with which the condition can be recognized. It must not be forgotten that the number of people taking amidopyrine is immense and that only a very small percentage of these show any symptoms of toxicity. In our experience, the percentage is smaller than in the case of acetyl salicylic acid. The symptoms, however, when they do appear, are somewhat more severe in the case of amidopyrine.

On the other hand, Herz,[20] who states that amidopyrine should not be classified as a benzene ring derivative, but as a pyrazolon derivative and therein lies its danger, concludes his article by stating that the dangerous character of amidopyrine has now been established beyond any reasonable doubt and that it should be banned by the medical profession.

In a preliminary report, Hoffman, Butt and Hickey[14] state that they have definitely shown that amidopyrine has an effect on the hematopoietic system of rabbits. They continue that "the common factor in amidopyrine, dinitrophenol, benzene, arsphenamine or the oxybenzoic acid and hydroquinone, all of which have produced neutropenia experimentally or clinically, is the benzene ring." Whether the latter is the actual toxic agent in the production of neutropenia, needs further investigation.

It seems unwarranted to condemn a drug that has been found useful for so many years without a definite and clear reason for so doing. Whether there be an individual susceptibility in the nature of an allergic reaction (Pepper[48]) or whether the cases be simply based on the law of chance association, must be determined. From our observations, it would seem that there is an individual susceptibility and how these allergic states differ remains to be solved. From a study of the Case I. D., reported here, who had taken both amidopyrine and antipyrine methyl amino methane sodium sulphonate without any effect before tonsillectomy, but who, when she received antipyrine methyl amino methane sodium sulphonate again, months after the operation, developed agranulocytosis, it might appear that the lymphatic tissues of the body had something to do with this susceptibility.

SUMMARY AND CONCLUSIONS

1. Thirty grains of amidopyrine have been given daily to each of 32 patients over periods extending from two weeks to three months and their granulocytic counts followed. There has been no marked change in any of the counts.

2. In other patients these drugs have been used over a period of four years and no dangerous symptoms have been noticed.

3. The absence of any ill effects in these cases may be due to a routine followed in our clinic, i.e., to withdraw any drug as soon as any symptoms of toxicity, as indigestion, lack of appetite, or dizziness, arise. It would seem that if this simple procedure were followed, there would be no need of frequent blood counts.

4. Skin tests with amidopyrine have been done on 50 individuals, one of whom had agranulocytosis and one of whom was quite sensitive to the drug. The only positive reaction was obtained in the last mentioned case.

5. Amidopyrine, in large doses, was administered to five patients on the ward. Four of these died. There was a differential drop in the granulocytes after each administration of the drug. Amidopyrine may have been responsible for this change in the white cell count.

6. Twenty cases of agranulocytosis are reported. Nine of these patients were known to have taken amidopyrine and one had taken antipyrine methyl amino methane sodium sulphonate.

7. The effect of neosalvarsan on the total and differential white counts of a patient with agranulocytosis is shown to be dangerous.

8. In two cases of polycythemia vera, phenylhydrazine stimulated rather than depressed the white cells.

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GUANIDINE-LIKE SUBSTANCES IN THE BLOOD*

IV. Blood Guanidine in Patients With Parathyroid Deficiency and With Idiopathic Tetany

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THE similarity of the symptoms of parathyroidectomy and guanidine poisoning very early led to the hypothesis that the symptoms of parathyroid tetany might be due to an accumulation or increased formation of guanidine in the body. In 1912, Koch,[1, 2] using a complicated method based on the one first described by Archelis,[3] claimed to have shown that there was a definite increase in the guanidine excretion of parathyroidectomized dogs. In 1917, Burns and Sharpe,[4] using a similar method, corroborated these findings. Paton and Findlay[5] also pointed out that the symptoms of parathyroidectomy are identical with those of guanidine poisoning. However, in 1918, Bauman and Ingvaldsen[6] showed that the methods previously used for the guanidine determination in urine, converted both creatinine and creatine into guanidine bases, and for this reason pointed out that all of the previous work was of little or no value.

In 1920, Findlay and Sharpe,[7] using a method whereby the guanidine in urine is precipitated with picric acid, confirmed their previous findings. In 1925, Sharpe[8] modified his picric acid method, and Kühnau,[9] using this method, together with the obsolete method of Archelis and a zinc precipitation procedure of his own, also appeared to show an increased excretion of guanidines in parathyroid tetany. However, about this time Greenwald[10, 11] showed that the solubilities of the picrates of creatinine, guanidine, and the methyl derivatives of guanidine are practically the same, and that a separation of these substances by picric acid precipitation is utterly impossible. White[12] upheld Greenwald's contentions. The observations of Greenwald and White clearly indicate that the results, obtained with Sharpe's methods and its modifications, are of little or no value.

Finally Küen,[13] in 1927, using a method whereby the guanidine in urine is precipitated with pierolonic acid, failed to show the presence of any guanidine in the urine of normal or parathyroidectomized dogs. He admitted, however, that his method was not sensitive for small amounts of guanidine.

In 1927, Major, Orr and Weber,[14] using their new blood charcoal method, failed to find any definite increase in the blood guanidine of parathyroidectomized dogs. The results of Raida and Liegmann,[15] von Beznák and Szeöke[16] and, very recently, Saunders[17] confirm these findings. Berenesy and Sinhobel[18] failed to find a close resemblance between the symptoms of guanidine poison-

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ing and of parathyroid tetany (in dogs), and Swingle and Nicholas[19] observed that the administration of guanidine to parathyroidectomized animals does not facilitate the production of tetanic symptoms. Bryan and Minot[20] reported first to have observed an irregular increase in the blood guanidine of parathyroidectomized dogs, but in the same year[21] they seem to have solved the whole problem by showing that the high values, occasionally observed in parathyroid tetany in animals, are explained on the basis of dehydration.

In fact, of the recent and more accurate work, only that of Remond and Colombies[22] and Traut and McFate[23] appears to show any relation between parathyroid activity and blood (or urine) guanidine. These investigators seem to have found that parathyroid extract lowers the blood guanidine in cases of hyperguanidinemia, and suggest that the concentration of guanidine in the blood may be partly regulated by parathyroid activity.

It might be well to add that Minot and her coworkers[24, 25] and also Schumaker[26] have found increased blood guanidine in tetany of children, but do not connect it with parathyroid activity. A number of investigators[27, 28, 29, 30] have observed hyperguanidinemia in epilepsy and other neurologic states, but suggest no relationship between the hyperguanidinemia and the parathyroid glands.

Summing up all the results obtained previous to this time, it is very doubtful if there is any significant change in the concentration of guanidine in the blood (or excretion in the urine) in parathyroid tetany.

EXPERIMENTAL

The method employed was the modified charcoal extraction method described in the first paper of this series.[31] The normal values obtained by this method average about 0.24 mg. per 100 c.c. of whole blood, with a range of from 0.18 to

TABLE I

BLOOD GUANIDINE IN PARATHYROID DEFICIENCY AND IDIOPATHIC TETANY
(Figures in mg. per 100 c.c.)

CASE	AGE AND SEX	UREA NITROGEN	SERUM CA	SERUM P	BLOOD GUANIDINE	DIAGNOSIS
1	47F	10	7.8	4.7	0.25	Parathyroid tetany
2	29F	11	8.2	4.0	0.22	Parathyroid tetany
3	43F	11	8.2	4.3	0.26	Parathyroid tetany
4	45F	12	9.5	4.5	0.32*	Parathyroid tetany
5	34F		9.5	4.1	0.22	Parathyroid tetany
6	49F	10	7.7	4.4	0.28	Parathyroid tetany
7	35F	11	9.1	5.4	0.23	Parathyroid tetany
8	28F	10	9.0	4.5	0.26	Parathyroid tetany
9	30F	12	10.0	3.3	0.17	Idiopathic tetany
9	30F	12	10.0	3.5	0.18	Idiopathic tetany

*This value is slightly high due possibly to the fact that the blood sample stood over night.

0.28 mg. In Table I are presented the results of blood guanidine determinations on 8 human cases of parathyroid tetany (accidentally produced by subtotal thyroidectomy), and one case of idiopathic tetany. All of the patients with parathyroid deficiency showed definite tetanic symptoms and were being given calcium salts. With some of the patients, over a year had elapsed since the operation. The serum calcium concentration is at the lower limits of normal or below normal, and the inorganic phosphate tends to be increased in a like manner. It is quite obvious that the blood guanidine concentration is essentially normal in all cases.

The results of this work seem to indicate that there is no definite increase in the blood guanidine of individuals showing parathyroid tetany, and make it appear very doubtful that the symptoms of parathyroid insufficiency are produced by increased guanidine in the blood or tissues.

One patient with idiopathic tetany showed a normal serum calcium and phosphorus concentration, and a blood guanidine value at the lower limit of the normal range. A second determination made several days later gave similar values.

SUMMARY

1. Studies on 8 cases of parathyroid tetany (following subtotal thyroidectomy) showed essentially normal values for blood guanidine.

2. One case of idiopathic tetany showed a blood guanidine concentration at the lower limits of the normal range.

We wish to take this opportunity to express our appreciation to Drs. Roy D. McCullough and E. J. McCullough of the Cleveland Clinic for their efforts in obtaining the blood samples, upon which the determinations described in this paper, were made.

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EXPERIMENTAL STUDIES ON LONG-CONTINUED ADMINISTRATION OF BISMUTH*

Summary Form

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STUDIES were made of morphologic blood elements, of the distribution of bismuth in the tissues, and of associated pathologic changes in tissues, in rabbits injected with bismuth. Young healthy rabbits were given bismuth at a moderate level dosage of 5 to 10 mg. per kg. body weight weekly for a long period of sixteen months. These were compared with other rabbits treated for shorter periods of two to fourteen days at high dosage of 50 to 100 mg./kg. weekly, and eight to twelve weeks at intermediate dosage of 10 to 30 mg./kg. weekly. The injection site was varied to allow time for absorption. Bismuth salicylate in oil was injected at weekly intervals, or bismuth sodium thioglycollate in water twice weekly. The animals died or were killed for examination.

I. Distribution of Bismuth in Tissues.—Although in the whole group the kidneys had a higher bismuth concentration, there was actually more bismuth in the liver. With an average liver weight of 60 gm., and the two kidneys 20 gm., the average amount in the individual animal's liver for the short period was 0.678 mg. and kidneys 0.536 mg.; for the intermediate period, liver 1.296 mg., and kidneys 0.854 mg.; for the long period, liver 0.630 mg., and kidneys 0.314 mg.

The renal bismuth, piled up in the epithelium of the convoluted tubules, is probably deposited there during concentration of the urine. There is no reason to believe this deposit is absorbed into the blood again. Desquamation of bismuth impregnated epithelium carries most of it down in the urine. With higher bismuth deposit cell death and desquamation are more rapid. Likewise, that accumulated in liver epithelium is on its way out of the body. The gallbladder bile in most animals contained bismuth, which reached a concentration of 6.5 mg. per cent in one animal. The major mobile deposit of bismuth is in the bones, as occurs with other heavy metals.

Injected muscles contained much of the insoluble bismuth which remained unabsorbed for indefinite periods. The soluble form disappeared rapidly. Other skeletal muscles contained only a trace of bismuth, while heart muscle contained appreciably more. Only traces also were in the lungs and brain, and none in the eyes.

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Newborn animals from bismuth-treated mothers contained bismuth, thus confirming transplacental passage. No bismuth was found in hyperplastic milk filled breast tissue of the mother.

Long-continued bismuth injection did not affect fecundity of animals, nor the viability of the young. Nor was the growth of young affected while nursing from a bismuth injected mother.

II. Pathologic Changes in the Tissues.—Tissues were formalin fixed, sectioned, and stained with hematoxyline-eosin, or with Van Gieson's stain when indicated.

The oil-suspended bismuth salicylate remained incompletely absorbed for long periods. Considerable muscle necrosis was caused by it, and marked polynuclear exudation, these often massing to form "chemical" abscesses. Scar formation was extensive, with "lakes" of oil enclosed.

The bismuth thioglycollate in water, absorbed rapidly, caused slight muscle injury with a few mononuclear cells such as gather at a point of mechanical trauma of muscle. Little or no scarring resulted.

No change was found in the liver, ascribable to bismuth, except marked cloudy swelling in the acute cases. In chronic cases, the slight periductal fibrosis and lymphocyte collections found were identical with those in untreated rabbits.

Heavy calcium deposits were found in degenerated areas of the kidneys in the acute and intermediate group. Also there were small calcium deposits in some of the kidney artery walls, and in degenerated heart muscle fibers in a few cases. These are the tissues most frequently calcified following necrosis from toxemia.

The kidneys had extensive necrosis of the epithelium of the convoluted tubules in the acute cases. To this was added, in the intermediate groups atrophy of the entire units affected, and diffuse fibrosis. In the chronic group there were scattered small cortical scars. Microscopic examination disclosed these as small bands of connective tissue extending across the cortex with atrophy of the tubules and glomeruli in the affected areas. Lymphocytes gathered in and about these scars, and elsewhere in the interstitium. Kidney tissue otherwise appeared unchanged. This lesion is identical with the spontaneous nephritis in rabbits.

The other body structures exhibited no significant morphologic change.

III. Morphology of the Blood.—Since syphilis invades the hemopoietic structures, any treatment affecting the disease may alter the blood picture, without necessarily having any primary influence on hemopoiesis. This accounts for the varied reports concerning the effects of bismuth on the blood. Using healthy young animals the attempt was made to evaluate the effect of bismuth on the formed elements of the blood. Regular counts and stained preparations were made during the period of bismuth injection.

The erythrocyte picture varied within a moderate range, without reference to the amount or form of bismuth given just as in normal animals.

Hemoglobin concentration tended to vary in the same direction as the red cells, and in fairly proportional amounts. Animals seemed to show individual stable levels, from a high of 90 per cent of hemoglobin or more with upwards of 7 million red cells to a low of 65 per cent hemoglobin and 4½ million red cells.

Basophilic stippling was not found in any blood.

Reticulocytes are present in appreciable numbers in normal rabbit blood, up to 4 per cent of the red cells and no alteration was produced by treatment.

There were fairly constant increases of the leucocytes with both forms of bismuth, both polynuclears and lymphocytes being affected. Normal rabbit blood has lymphocytes predominant, often as high as 75 per cent of the total.

With the higher counts of bismuth treatment, up to 26,000, the proportion of polymorphonuclears was increased, occasionally to the reverse of the normal count. Changes in the numbers of other white cells were insignificant.

MUMPS*

The Incidence of Palpable Splenic Enlargement and of "Complications," and Their Relation to Salivary Gland Involvement as Evidence That the Disease Is a Systemic Infection

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THE fact that mumps is rarely fatal probably accounts for the lack of general interest in the disease. A majority of the reports in the literature are the result of sporadic interest aroused because some of the more rare manifestations have been observed or a high incidence of "complications" has been encountered. Our interest was aroused when we observed a white male, aged seventeen years, who entered the University Hospitals with no other complaint than swelling of the left side of the neck of two days' duration. The swelling was found to extend over the left anterior triangle of the neck, and to include the submaxillary gland and the cervical lymph nodes. The area was tender and definitely demarcated, but was not fluctuant. The overlying skin was reddened, and warmer to touch than that adjacent to it. There was neither enlargement nor tenderness of the parotid glands, no redness of the openings of Stensen's ducts, and no enlargement of other lymph nodes. The spleen was palpable 2 cm. below the costal margin at the height of inspiration. The temperature was normal. Examination of the blood revealed only a relative lymphocytosis. The diagnosis was not evident until swelling of the right parotid gland appeared four days later, and a history of recent exposure to mumps was elicited. The subsequent course, which was typical for the disease, confirmed the diagnosis of mumps.

Involvement of the overlying skin has been recorded in the literature, but palpable splenic enlargement is not mentioned in the textbooks. Radin[1] in a study of 5,756 cases noted a palpable spleen in only one and this patient gave a history of previous attacks of malaria. In practically all of Capitan's[2] 700 cases the spleen was found to be enlarged to percussion, but nothing was said about its being palpable.

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TABLE I

CASE	AGE YR.	SEX	INITIAL MANIFESTATIONS				LATE MANIFESTATIONS					OTHER DISEASES PRESENT
				DURATION AT EXAMINATION, DAYS	SPLEEN			OCCURRENCE, DAY OF ILLNESS	EXAMINATION, DAY OF MANIFESTATION	SPLEEN		
					PALPABLE	DURATION, DAYS				PALPABLE	DURATION, DAYS	
1	17	M.	Left submax.	2	Yes	–	R. parotid R. submax.	6	–	–	–	None
2	21	M.	R. parotid R. submax.	3 5	No Yes	–	L. parotid	4	2	Yes	–	None
3	14	M.	Bilat. parotids	10	No	–	–	–	–	–	–	Diabetes
4	21	M.	Bilat. parotids L. submax. ? encephalitis	3	No	–	–	–	–	–	–	None
5	29	F.	Bilat. parotids	3	No		Discharged on day of admission					None
6	19	M.	L. parotid	6	No	–	Bilat. submax. and sublingual	6	2	No	–	None
7	23	M.	R. parotid	6	No	–	L. parotid R. orchitis and epididymitis ? pancreatitis	7 8 11	–	–	–	None
8	18	M.	Bilat. parotids	2	Yes	–	–	–	–	–	–	5 days Postoperative appendectomy
9	12	F.	Bilat. parotids	8	Yes	–	Bilat. sublingual	?	?	Yes	–	4 days Postoperative appendectomy
10	16	F.	Bilat. parotids	5	No	–	Bilat. submax.	2	3	No	–	Osteomyelitis Os calcis
11	9	M.	R. parotid	2	Yes	2	L. parotid Encephalitis	4 7	1 1	No No	– –	Tendon Transplant. Old Ant. Polio.
12	14	M.	Bilat. parotids	2	Yes	–	Encephalitis	7	3	No	–	

TABLE I—CONT'D

CASE	AGE YR.	SEX	INITIAL MANIFESTATIONS			LATE MANIFESTATIONS					OTHER DISEASES PRESENT
				DURATION AT EXAMINATION, DAYS	SPLEEN			OCCURRENCE, DAY OF ILLNESS	EXAMINATION, DAY OF MANIFESTATION	SPLEEN		
					PALPABLE	DURATION, DAYS				PALPABLE	DURATION, DAYS	
13	10	M.	Bilat. parotids (mild)	4	No	–	None	–	–	–	–	Psoriasis
14	19	F.	Encephalitis L. submax.	8	–	–	R. parotid	4	4	Yes	–	None
15	30	F.	L. parotid	4	No	–	R. parotid	3	2	No	–	None
16	19	F.	R. parotid R. submax.	1	Yes	3	None	–	–	–	–	Latent Syphilis, 2 Days Post-Partum
17	20	F.	L. parotid	2	Yes	6	R. parotid Encephalitis L. submax.	5 6 8	1 1 1	Yes Yes Yes	4 3 1	None
18	19	F.	Bilat. parotids	1	Yes	1	None	–	–	–	–	None
19	26	F.	L. parotid (receding) R. parotid (increasing)	3 4 5	No Yes No	1	None	–	–	–	–	None
20	17	M.	L. parotid (mild)	2 3	No No	–	None	–	–	–	–	None
21	26	M.	R. parotid	7	–	–	L. parotid R. orchitis R. epididymitis ? Encephalitis	4 6 6 7	4 2 2 1	Yes Yes Yes Yes	3	None
22	23	F.	Bilat. parotids	3 4 5	No	–	None	–	–	–	–	None
23	26	M.	Bilat. parotids	5 7	No No	–	R. orchitis Encephalitis	9 11	4 2	Yes	1	None
24	19	F.	Bilat. parotids	2	Yes	2	R. submax.	3	1	Yes	1	None
25	32	M.	L. submax.	–	–	–	R. parotid Bilat. orchitis Pancreatitis Encephalitis	3 7 10 12	1 1 1 1 2 4	No No No No No No	–	None

Twenty-five patients with mumps who were admitted to the isolation ward of the University Hospitals during two years were examined particularly for palpable splenic enlargement. The first nine were examined only once, whereas the remainder were seen at frequent intervals in an attempt to correlate splenic enlargement with other manifestations.

It will be noted in Table I that the spleen was enlarged sufficiently to be palpable in 14 of the 25 patients.

A study of the records of 75 additional unselected cases revealed that during the routine physical examination the spleen was found to be palpable in 5. The organ more commonly enlarges during the first few days of the disease, but may enlarge at any stage. It was palpable in 12 of 19 instances on or before the third day, persisted throughout the illness in one (Case 17, Table I), and enlarged on the twelfth day in another (Case 23, Table I). Palpable enlargement usually persists for only one or two days. It usually accompanies salivary gland involvement, but may enlarge with other manifestations (Case 23, Table I). The possibility that latent syphilis was the cause of splenic enlargement in one case is excluded by the fact that the organ ceased to be palpable as the mumps subsided.

The high incidence of palpable splenic enlargement, and the fact that the spleen may be palpable with any of the manifestations of mumps strongly support the concept advocated by Albright,[3] Montier,[4] Laveran,[5] Montgomery,[6] and Stengel[7] that the disease is a general infection with the so-called complications appearing only as varying manifestations of the disease. This concept is further supported by several observations[8] that mumps orchitis, oophoritis, and meningoencephalitis may occur prior to, coincidental with, or even without, involvement of the salivary glands.

There has been, however, no attempt to ascertain the relative frequency with which the various manifestations appear in relation to involvement of the salivary glands. The records of 100 unselected cases of mumps were therefore analyzed to ascertain the incidence of the "complications," and the relation of the onset of these manifestations to the initial salivary involvement. The patients in this group were largely university students and members of the hospital staff. The majority were young adults, and the sexes were about equally represented.

"Complications" were noted in 30 per cent of the cases. There occurred definitely 41 nonsalivary manifestations and an additional 10 which were doubtful in the 30 cases. The seeming discrepancy of the figures in Table II, which

TABLE II

OTHER MANIFESTATIONS	PRIOR TO SALIVARY GLAND INVOLVEMENT	SIMULTANEOUS WITH SALIVARY GLAND INVOLVEMENT	FOLLOWING SALIVARY GLAND INVOLVEMENT	TOTAL INCIDENCE PER CENT
Orchitis	1	8	11	18 (43 per cent adult males)
Encephalitis	2 and 1 ?	1 and 1 ?	8 and 4 ?	11 to 17
Pancreatitis	1	1 and 1 ?	5	7 to 8
Oophoritis	0	1 ?	3	3 to 4 (5.3 to 7.1 per cent adult females)
Prostatitis	0	0	2	2 (5 per cent adult males)
Conjunctivitis	0	1 ?	1 ?	0 to 2
Total	4 and 1 ?	10 and 4 ?	29 and 5 ?	41 plus 10 ?

shows the incidence of the "complications" and the relation of their onset to the initial salivary involvement, is the result of bilateral orchitis in two cases which appeared on one side early and on the other side late in the disease. The incidence of orchitis in this study (43 per cent of adult males) agrees with other investigations,[5B] but the incidences of pancreatitis (7 to 8 per cent), of encephalitis (11 to 17 per cent), and of oophoritis (5.3 per cent of adult females) are greater than those generally reported. The frequency of pancreatitis does, however, agree with that reported by Brahdy and Scheffer.[9] The incidence of so-called complications varies in different epidemics, but our cases were not confined to any one epidemic because they were distributed over a period of six years.

It will be noted from Table II that the "complications" were the initial manifestations of the disease in 4 per cent of the cases, and accompanied the initial salivary gland involvement in 10 per cent. These lesions were, therefore, either the first or one of the first manifestations of the disease in 46.6 per cent of the cases in which they occurred. Instances of "complications" without salivary manifestations were not encountered in this study. The high incidence of involvement of other organs prior to, or coincidental with, involvement of the salivary glands is additional support to the contention that the disease is a systemic infection.

It is generally conceded that two or more attacks of mumps may occur, but that they are rare. In our series there were three who had two attacks, and one who had three attacks, an incidence of 4 per cent. It is also generally thought that the disease does not involve the same salivary glands twice. The same salivary glands were, however, involved in each of the two attacks in three cases. The other patient had three attacks of bilateral parotitis, two of which were accompanied by bilateral orchitis, and the last attack was accompanied by pancreatitis.

An occasional person is encountered who has never had mumps, but who has been exposed to the disease on several occasions. It appears possible that some of these persons may have had the disease without any localizing manifestations. This possibility is supported by the highly contagious character of the disease, by the fact that the disease may occur without the salivary glands becoming involved, and if the disease is a systemic infection, which our evidence supports, localizing manifestations need not occur.

SUMMARY

A study of 25 cases of mumps for enlargement of the spleen revealed that it was palpable in 14 instances. A review of 75 additional cases showed that enlargement was noted on routine physical examination in 5 instances. The enlargement is more common during the first few days of the disease, but may appear later. It most frequently accompanies salivary gland involvement, but may appear with other manifestations.

The so-called complications (other manifestations) were noted in 30 per cent of the cases. Four per cent of such manifestations appeared before, and 10 per cent coincidental with, involvement of the salivary glands.

Recurrent attacks of mumps were noted in 4 per cent of our cases, all of which had involvement of the same salivary glands in each attack, and one case had other manifestations during each of three attacks.

The findings support the contention that mumps is a generalized infection. It is suggested that the disease may occur without localizing manifestations.

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SUCCESSFUL TREATMENT OF PERSISTENT EXTREME DYSPNEA "STATUS ASTHMATICUS"

Use of Theophylline Ethylene Diamine (Aminophylline, U. S. P.) Intravenously*

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With the Help of John Martin, M.D., as a Senior N. Y. A. Student Assistant

ACUTE dyspnea is a medical emergency, particularly when persistent and not responsive to epinephrine or morphine. Any preparation which will relieve the acute respiratory distress under such conditions fairly regularly, promptly, and with reasonable safety deserves careful consideration.

The measures generally employed depend to some extent upon the underlying pathologic physiology and the accompanying complications. In general practice as well as in the emergency service of a large metropolitan hospital one is likely to have presented acute dyspneas of various types. The great majority of dyspneic patients suffer from cardiovascular disorders, particularly acute congestive left ventricular failure. However, acute bronchopulmonary conditions are responsible for a large number of the most severe types of acute dyspnea. These at times are not quieted by morphine and have become refractory to epinephrine and adrenalin hypodermatically. Such are often designated as "adrenalin fast status asthmaticus" cases.

Morphine sulfate has long been the drug of choice in most types of dyspnea. It is the most indispensable drug in the physician's possession. The dramatic life-saving effect, the effort-sparing and quieting action of morphine in patients in acute congestive failure, acute pulmonary edema, and paroxysmal nocturnal dyspnea are well known. Cheyne-Stokes breathing may, however, be aggravated, with the apneic periods greatly lengthened, by morphine. In status asthmaticus morphine is palliative at best, may actually depress the respiratory center dangerously and contribute to pulmonary edema if given repeatedly in large doses.

Epinephrine hydrochloride (adrenalin) and ephedrine hydrochloride are, of course, usually most efficacious in bronchial asthma but not infrequently one encounters cases in which the drugs fail to relieve the dyspnea. Such patients are sometimes designated as "adrenalin-fast status asthmaticus" cases. Under such circumstances one must resort to more heroic methods, such as the intravenous injection of small doses of 0.1 to 0.2 c.c. of 1-1,000 adrenalin; 10 c.c. cal-

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cium chloride 10 per cent solution; calcium gluconate 10 per cent solution; or 25 to 50 c.c. of hypertonic 50 per cent glucose solution. All of these have been used with some success.

We have found, however, that theophylline ethylene diamine (aminophylline, U. S. P., euphyllin, metaphyllin, thephyldine) in doses of 0.48 gm. (7½ grains) in 10 c.c. saline injected slowly is most uniformly successful with least serious reactions. We have found aminophyllin effective promptly and totally in fully 75 per cent of the cases of "status asthmaticus" in which we have used it. In some cases the relief afforded has not been immediate and complete but in most of these it has been both delayed and partial. In our hands the drug has rarely been totally ineffectual.

Our interest in this seemingly neglected and apparently important therapeutic action of aminophylline, U. S. P., was aroused during the progress of some clinical investigations (1) on the comparative diuretic effectiveness of various intravenously injected xanthines or purines, and mercurials begun in the Charity Hospital in New Orleans and continued in the John Sealy Hospital in Galveston.[1] We were often struck by the spectacular relief of urgent dyspnea as well as cardiac pain that resulted from the intravenous introduction of aminophylline in 0.48 gm. (7½ gr.) doses. We frequently noted the immediate disappearance of Cheyne-Stokes breathing following the intravenous introduction of aminophylline and have tried to explain the improvement on the basis of increased blood flow through the medulla but the response was so prompt that such could hardly be the case. It may be the result of a direct action of the drug on the cells of the respiratory center. Paroxysmal nocturnal dyspnea, cardiac asthma, have been noted to subside quickly after intravenous aminophylline. Again this may be response to improved circulation but it may also be central effect. Similar subsidence of nocturnal attacks may, however, follow profuse diuresis of salyrgan origin. Some of our patients whom we considered to be suffering from congestive heart failure also had pulmonary signs suggestive of asthmatic bronchitis and sometimes it was difficult to determine which was primary.

In one early series (1931) studied with Dr. Wm. L. Marr, of 5 elderly patients suffering from acute respiratory infection, asthmatic bronchitis and acute vesicular emphysema, 4 were treated with the usual antispasmodics and all died, while the fifth one treated with aminophylline (0.48 gm. diluted to 10 c.c.) intravenously alone survived.

We have found reports of respiratory improvement following the administration of aminophylline. Respiratory stimulating effects pulmonary circulatory augmenting effect, and the smooth muscle relaxing or possible bronchial clearing or effects have, however, not been commented upon in our literature.

Vogl[2] has reported on the value of xanthines, especially aminophylline, in respiratory disorders of central origin, particularly Cheyne-Stokes breathing in patients with general arteriosclerosis; and in central dyspnea resulting from pulmonary congestion and cardiac asthma. These conditions were relieved by the intravenous administration of 0.48 gm. (7½ gr.) of aminophylline.

Freud[3] has used aminophylline successfully, by rectal administration, in infants with periodic breathing of capillary bronchitis, in pneumonias of childhood, and in the periodic breathing of encephalitis, all of which are considered central disturbances of respiration.

Bock[4] demonstrated uniform dilatation of the blood vessels of the isolated dog's lung perfused under constant pressure as well as of the dog's extremity and kidney after the injection of 0.5 to 1.0 gm. of theophylline. The effect appeared within one to five minutes, usually within two minutes and lasted from one-half hour to one and one-half hours. The maximum effect was present in fifteen to twenty-one minutes. The effects of xanthines on the cerebral vessels and on the bronchial musculature have not been experimentally established.

In the report of the referee for the Council on Pharmacy and Chemistry of the American Medical Association (J. A. M. A. 94: 1306, 1930), there is abstracted the important literature up to that time with reference to the therapeutic effects of the xanthine derivatives. It is stated that according to Eppinger and Hess[5] the coronary artery dilated when placed in a weak solution of caffeine. Sollmann and Pilcher[6] found that the vasodilation resulted from peripheral inhibition of vasoconstriction. Meyer,[7] Hedbom,[8] and Loeb,[9] in perfusing isolated hearts with xanthine solutions, noted increased coronary flow which they considered to be due to vasodilation while other investigators, Sakai and Saneyoshi,[10] believed the increased coronary output to be the result of a rise in blood pressure. The latter experimenters[10] found that theobromin gave a greater augmentation of coronary flow. Heatheote[11] added that theophylline, as well as the other preparations, was an active vasodilator for coronary arteries, an action that he concluded was probably muscular in origin. Smith, Miller, and Graber[12] found experimentally that theophylline was the most effective of all the xanthines when given in the minute doses in which it might be found in the blood after administration by mouth. There has been much indirect personal clinical confirmation of this by the relief of angina pectoris following the ingestion and particularly injection of the drug. Blood pressure lowering has not been conspicuous and the value of the xanthines for this purpose has not been established. The council decided that the claims for diuretic action and myocardial stimulation and possibly vasodilating action were permissible. There was no mention of any theophylline effect on respiratory disorders, on the pulmonary circulation, or on the bronchial musculature in the earlier studies.

OUR PRESENT STUDIES

For the past six years we have been using the intravenous injection of theophylline in doses of 0.48 gm. in 10 c.c. of saline for the relief of persistent extreme dyspnea, "status asthmaticus" and "adrenaline fast" asthmatics. We have always given the solution slowly through a very small needle in order to avoid unpleasant reactions. Such reactions, however, have occurred fairly frequently in spite of all precautions but have been only slightly unpleasant and never serious, in our experience. Most of the patients complain of a momentary feeling of heat in the skin, particularly of the face, burning in the eyes, some-

TABLE I

EFFECT OF AMINOPHYLLINE INTRAVENOUSLY IN STATUS ASTHMATICUS IN HOSPITAL PATIENTS

CASE	NAME, COLOR, SEX, AGE, HOSPITAL NUMBER	ADDITIONAL DIAGNOSES	PREVIOUS MEDICATION (EMERGENCY ROOM)	DURATION OF ASTHMA DURATION OF ATTACK	DOSE GM.	RELIEF IMMEDIATE	INTERVAL UNTIL NEXT ATTACK	REACTION TO DRUG	REMARKS
I	W. E. W. M., 79 205449	Chronic emphysema. Fibroid tuberculosis.	Adrenalin, ephedrine, morphine, digitalis. Ca Cl_2, Ca gluconate intravenously. Catarrhal vaccine.	25 years. Hours to 10 days.	0.48	Prompt. As short as eight minutes.	Usually a year. Sometimes only eight months.	Burning sensation in eyes. Headache.	After first treatment, blood pressure fell from 200/130 to 135/75, and remained at the low level. Vital capacity increased as much as 750 c.c.
II	R. L. W. F., 13 39381	Bronchopneumonia.	Adrenalin, to ♏ L. Atropine, novatropine. Morphine. Glucose I-V. Phenobarbital. Ephedrine pantopon. Oxygen tent.	7 years. Hours to 3 days.	0.24	Sometimes in only ten minutes, other times, definite but not spectacular.	Long enough to be removed to Arizona, away from sensitive zone.	Heat over body. Nausea. Could not sleep for hours.	Subsequently moved to Arizona, where patient has been free from symptoms. Relieved in extremis by aminophylline, when all else had failed.
III	A. G. G. W. F., 37 4981	Urticaria. Diaphragmatic hernia.	Ephedrine, atropine, codeine, adrenalin, morphine, novatropine. Glucose, calcium gluconate.	11 years. Hours to days.	0.24 and 0.48	"Complete before injection was finished."	3 months.	Warmth. Nausea. Vomiting.	After patient had become "adrenalin-fast," aminophylline relieved her many times.

TABLE I—CONT'D

CASE	NAME, COLOR, SEX, AGE, HOSPITAL NUMBER	ADDITIONAL DIAGNOSES	PREVIOUS MEDICATION (EMERGENCY ROOM)	DURATION OF ASTHMA DURATION OF ATTACK	DOSE GM.	RELIEF IMMEDIATE	INTERVAL UNTIL NEXT ATTACK	REACTION TO DRUG	REMARKS
IV	H. H. T. W. M., 25 (?) 34900	Maxillary sinusitis.	Adrenalin (large amount). Ca. gluconate & glucose. Oxygen tent.	4 years (only when in Galveston). Hours to days.	0.24 and 0.48	"Greatly relieved."	Moved after graduation.	Slight flushing, momentary increased constriction of chest, sensation of warmth and of "something let loose."	Senior medical student. Reported no further distress after graduation.
V	J. C. M. G. W. M., 66 206037	Extensive old bilateral tuberculosis.	Adrenalin.	8 months. Hours to days.	0.24 and 0.48	Complete relief in thirty minutes.	Two weeks.	"Peculiar" precordial feeling.	Loss of 30 pounds in the eight months. Sputum contained tubercle bacilli.
VI	W. E. R. M., 63 46007	Asthmatic bronchitis.	Codeine, novatropine. Phenobarbital. Ephedrine. Atropine, digitalis.	7 years. Several hours.	0.48	Complete symptomatic relief.	3 months (?).	Excitation. Sleeplessness.	While symptoms were relieved, vital capacity, pulse, and blood-pressure were not affected.
VII	B. C. W. 32683	Maxillary sinusitis. Emphysema.	Ephedrine. Adrenalin. Typhoid vaccine.	17 years. Hours to "2½ years, day and night, summer and winter."	0.48	Marked relief at first, but in time, only "improvement."	1 month. Patient moved.	None noted.	Patient first became refractory to adrenalin, after which aminophylline afforded relief, then patient became refractory to latter drug, and adrenalin again became effective.

times there is a sense of constriction in the chest, occasionally nausea and vomiting and in very rare instances cerebral manifestations, twitching, convulsion, and coma. There have not, however, been any fatalities as a result of the injection of this drug in a hundred or more experiments and no report of death from intravenous injection of theophylline has been found in the literature.

We have not kept a cross index of all of our cases of respiratory distress treated with intravenous theophylline and we are certain many injections were given that were not recorded, particularly in the emergency room. We have, however, found the records of 7 of our hospital cases and 9 out-patients that were so treated. Short abstracts of the histories of these will be set down to illustrate our results.

SOME CASES OF STATUS ASTHMATICUS TREATED BY THEOPHYLLINE ETHYLENE DIAMINE

Case 1.—W. E. (Hospital number 205449), a white male, aged seventy-nine years, had suffered from attacks of shortness of breath for twenty-five years. The attacks usually have been associated with respiratory infection during the cold damp months of January, February, March, and April. For about fifteen years he has been having attacks earlier in the fall and winter and later in the spring. Since 1930 he has been admitted to the hospital with severe attacks no less than 20 times, each time with the same symptoms and signs of asthmatic bronchitis with evidences of chronic emphysema and a roentgenologic diagnosis by Dr. J. B. Johnson of fibroid tuberculosis. He had generally been relieved by the use of adrenalin or ephedrine with or without morphine and occasionally digitalis was used and on rare occasions calcium chloride or calcium gluconate had to be given intravenously to get results.

At times, however, he has been refractory to all this therapy. Early on October 8, 1931 he had, in addition to dyspnea, a very severe attack of abdominal pain with vomiting, a blood pressure rise to 200/130, and electrocardiograms showing defective intraventricular conduction. At this time he was given theophylline ethylene diamine (thephyldine) 0.48 gm. diluted to 10 c.c. with saline intravenously, which promptly and completely relieved him of extreme dyspnea which was considered to be a part of his asthmatic bronchitis. His blood pressure ever after this attack was much lower, usually in the neighborhood of 130 to 140 over 70 to 80. This may have been an attack of coronary thrombosis.

He was given two courses of catarrhal vaccine but this did relatively little good. An exacerbation and exaggeration of the thoracic pulmonary distress followed each acute upper respiratory infection. All sorts of expectorants were used without avail and ephedrine, digitalis, theophylline e. d. (metaphyllin) tablets, 1½ gr. tablets were given regularly thrice daily by mouth. He was kept fairly comfortable on this regime and occasionally adrenalin had to be given hypodermatically.

At times he became refractory even to adrenalin and morphine and then 0.48 gm. of aminophyllin in 10 c.c. of normal saline was administered intravenously with, as a rule, prompt and complete relief with only a momentary burning sensation in his eyes as a reaction to the drug. On Oct. 7, 1932 his vital capacity rose from 1.75 liters, about 40 per cent of normal for him, to 2.2 liters and his blood pressure dropped to 128/60 to 119/58. This therapy was repeated Feb. 12, 1933, Feb. 13, 1934, Sept. 7, 1934, June 9, 1935. Calcium chloride 10 c.c. of a 10 per cent solution intravenously was tried on Sept. 13, 1935 and calcium gluconate 10 c.c. of a 10 per cent solution intravenously on Dec. 3, 1935 but aminophyllin was again resorted to on Dec. 6, 1935, and on May 6, 1936 when he had in addition to his dyspnea a severe precordial pain which was thought to be possibly coronary thrombosis but may well have been an interstitial mediastinal emphysema.

On Dec. 9, 1935, 3 gr. of aminophyllin by mouth t.i.d. kept him comfortable for a few days. He had been taking his regular oral medication of ephedrine and digitalis as

well as aminophyllin. Nevertheless, on Dec. 16, 1935 he came into the emergency room severely dyspneic and cyanosed. Adrenalin failed to relieve him and again 0.48 gm. of aminophyllin intravenously was promptly effective. On Oct. 10, 1936 he came in with an attack that had been persistent for ten days in spite of all medication including adrenalin. In addition to dyspnea and cyanosis, precordial pain and coughing were complained of and again 0.48 gm. of aminophyllin intravenously at 4 P.M. relieved him completely. He always felt the momentary burning sensation following the injection. On Jan. 22, 23, 24, and 25, 1937 the effect of the intravenous injection of 0.48 gm. of aminophyllin in 10 c.c. of saline on the vital capacity, blood pressure, respiration,and pulse were recorded.

During the seizure on Jan. 22, 1937, the vital capacity was 1,500 c.c.; the respiration rate, 24; the pulse rate, 96; and the blood pressure, 130/70. Eight minutes after injection he was completely relieved, had some burning in the eyes and headache, and his vital capacity had increased to 1,750 c.c.; his respirations dropped to 20; and his pulse, to 60 while his blood pressure remained unchanged.

Under similar circumstances on Jan. 23, 1937 with a vital capacity of 2,100 c.c.; respiration, 20; pulse, 64; and blood pressure, 110/60; aminophyllin relieved his distress and his vital capacity rose to 2,200 c.c.; his respirations to 24; and his blood pressure to 120/65.

Likewise on Jan. 24, 1937 in a severe attack his vital capacity was 1,750 c.c.; respiration 20, pulse 60, and blood pressure 110/60, while after relief by aminophyllin his vital capacity rose to 2,100 c.c. but no change was recorded in the respirations, pulse, and blood pressure.

And on Jan. 25, 1937 his vital capacity dropped to 1,500 c.c. with respiration rate 24, pulse rate 60, and blood pressure 120/68, while following aminophyllin the vital capacity rose to 2,250 c.c., the respirations and pulse rates remained unchanged and the blood pressure dropped to 110/60.

CASE II.—R. L. (Hospital number 39381), a school girl, aged thirteen, was first treated when she was eight years old, Oct. 5, 1932, for status asthmaticus. She had been under the care of Dr. Paul Woodard for some time, but he had not been able to find her sensitive to any of the ordinary allergens. Her attacks apparently followed each "norther" and were usually accompanied by an infection of the upper respiratory tract. On this day she had become much worse and failed to respond to adrenalin in 1 c.c. doses of 1/1000, atropine, novatropine, and morphine. All had failed to relieve her, and she went into collapse. She was given 20 c.c. of 25 per cent solution of glucose, intravenously and put under an oxygen tent. Then 1 c.c. containing 0.24 gm. of theophyllin e. d. was diluted and given intravenously following which she improved definitely though not spectacularly. She was discharged in a few days, but on Oct. 12, 1932 she again became refractory to all therapy which she was taking and was readmitted to the hospital and put on the same regime which she previously had taken and again 1 c.c. of 0.24 gm. of theophyllin e. d. was the only medication that brought any relief. She improved sufficiently to be moved to the southwest where she was entirely from of symptoms and in good health.

In the fall of 1936 she returned to Galveston. On Jan. 31, 1937 following a sinus infection she had a recurrence of her severe attack of asthma and was given phenobarbital and ephedrine and recovered. On Feb. 13, 1937 following another upper respiratory infection she was admitted to the hospital with fever of 103.8°, respirations 28, pulse 120, and a roentgenogram revealed evidence of bronchopheumonia with diffuse infiltration throughout both lungs, and an interlobar pleurisy between the middle and upper lobe on the right.

She had fever for only three days, and the respiratory distress continued in spite of the counterirritation to the chest, expectorants, sedatives, atropine and adrenalin. On Feb. 11, 1937 she went into a status asthmaticus and was given subcutaneously pantopon, 1/3 gr., atropine, 1/150 gr., and adrenalin minims 10. This was repeated six hours later but between these doses three injections of 15 minims each of adrenalin had to be given. She was most certainly adrenalin-fast. Oxygen therapy was instituted and she was given 20 c.c. of 50 per cent glucose intravenously. As this seemingly did no good and she was in extremis, intravenous aminophyllin administration was ordered but only a half dose of 1 c.c. or 0.24 gm. was diluted and injected. She complained some of a feeling of heat over the body and some nausea but within ten minutes she could breathe with comparative ease. She was

somewhat excited by the drug and although exhausted she could not go to sleep for some hours. Phenobarbital quieted her. The aminophyllin injection was repeated once more in the early morning hours. Improvement was rapid thereafter. Within twenty-four hours she was on her way back to Tucson, Arizona. She cleared up on the trip and remains free of trouble in Arizona.

CASE III.—A. G. G. (Hospital number 4981), a Mexican housewife, aged thirty-seven, first came under observation, Aug. 19, 1931 with a burn on the hand and asthma. She appeared in attacks in the clinic in 1926 and 1927 at which time ephedrine capsules of ¾ gr. each were successful in relieving attacks. In 1928 on one occasion she became refractory but was relieved after many capsules were taken and an injection of atropine and codeine sulphate. Her attacks usually began in the month of August and recurred each fall with greater intensity, particularly when the wind was in the north. After a few years she suffered attacks at other times in the year.

In 1929 a severe attack was relieved by adrenalin. She was seen at this time by Dr. Homer Prince who thought that there was a nervous basis rather than an allergic basis for her attacks since they were always worse during and after pregnancy. She had pain in the left chest on frequent occasions. Ephedrine and adrenalin seemed to relieve her of attacks during 1929 and 1930. In 1931 her attacks became more frequent in the spring as well as the fall. She had severe coughing as well and on one occasion she thought she pulled something loose inside of her chest. She showed the same symptoms, dyspnea and pain and signs as sonorous râles of asthmatic bronchitis and emphysema throughout both lungs on each admission. The roentgenogram revealed lungs that were clear except for considerable fibrosis in and about the hilar regions.

She was operated upon for bilateral salpingitis, appendicitis, and suffered from chronic pleuritis late in 1931. In 1932 she had an attack of rash and urticaria following the eating of raw oysters. On one occasion in the late fall of 1932 ephedrine was ineffectual and adrenalin 15 min. 1 c.c. and morphine 15 mg. relieved the attack. In 1933 0.0025 gm. of novatropine t.i.d. was used with some success to prevent the asthmatic seizures. In 1933 the attacks recurred about every two months. Atropine and morphine were frequently used along with 1.3 c.c. of adrenalin. She became "adrenalin-fast" during a prolonged attack in the autumn of 1933. One cubic centimeter of 0.24 gm. of theophyllin ethylene diamine intravenously was followed by some relief. Afterwards she again responded to adrenalin. During 1934 she was taking ephedrine religiously. Her blood pressure which had previously been normal, rose to 144/78, 187/88, 190/112, and 210/100. She began to have attacks of asthma at least once a month and often every two weeks. In 1935 she was given 1½ gr. of aminophyllin t.i.d. in addition to her previous therapy. Her attacks became less frequent, occurring only about once in three months. The asthma, however, did not clear up entirely. Examination of the lungs always showed râles, even between the severe attacks. The blood pressure continued at high levels. In 1935 it was 190/120, and in a severe seizure on Feb. 21, 1935 she was given intravenously 10 c.c. of calcium gluconate of a 10 per cent solution diluted in 50 c.c. of a 50 per cent solution of glucose which relieved the status asthmaticus. The blood pressure dropped to 148/80.

On Sept. 28, 1935 she came in to the hospital in an attack of status asthmaticus which had been continuous for two weeks in spite of the taking of 50 mg. (¾ gr.) capsules of ephedrine every four hours and an injection of 1 c.c. of adrenalin in the emergency room. Upon admission to the hospital her condition was considered to be grave and theophylline e. d. (aminophyllin) Searle's 0.48 gm. in 10 c.c. of saline was administered intravenously. The attack ceased before the injection was completed. She complained of a momentary feeling of warmth and nausea which was followed by vomiting. She left the hospital the next morning.

She continued to have recurrent minor attacks of dyspnea which were relieved by the ordinary medication. On Jan. 31, 1936 she had a rash with itching, burning, smarting, and swelling of the lower lip after eating eggs and bacon for breakfast. This was relieved by adrenalin. On Feb. 5, 1936 she had a similar attack and was placed on elimination

diet. She has had no recurrences of the rash but the dyspneic attacks have recurred. She was found to have a massive herniation of the stomach through the diaphragm into the left chest.

CASE IV.—H. H. T. (Hospital number 34900), a senior medical student, first came under observation in 1931 with sinusitis which required surgical drainage. He had never had any trouble before coming to Galveston to attend the medical school. Each autumn following each "norther" he would have asthmatic attacks. Various tests for sensitization were tried, and he was found to be slightly sensitive to house dust and marsh elder but not markedly so to either. The asthmatic seizures became longer and more severe each year.

During the latter part of his senior year, May 19, 1934, he began to have an attack following a spring "norther" and unlike most of his attacks he was unable to control this one with adrenalin. He had injected adrenalin in 1 c.c. doses at hourly intervals for about half a day before he sought admission to the hospital. He was extremely tremulous, dyspneic, orthopneic, and cyanotic. The neck veins were distended and auscultation of the chest revealed high pitched musical râles. Adrenalin injections were continued. Each administration caused him to vomit. Ten cubic centimeters of calcium gluconate of a 10 per cent solution was given in 100 c.c. of 25 per cent glucose solution which helped very little. The oxygen tent seemed to do little good. Finally theophyllin e. d. (metaphyllin 0.24 gm.) was given intravenously. This caused slight flushing, momentary increased constriction of the chest, a feeling of warmth and then "a feeling as though something let loose." He was able to expectorate a thick tenacious mucopurulent sputum and was greatly relieved. He seemed better able to make use of the oxygen tent which was continued for some hours. Dilaudid $\frac{1}{20}$ gr. was given and he went off into a peaceful sleep. On awakening he continued to vomit for some hours and developed a mild acidosis. The patient became jaundiced. Dr. D. P. Wall found nasal polyps and a maxillary sinusitis and took steps to shrink the nasal mucosa with adrenalin. The sinuses were drained, the polyps were removed and he improved. Upon graduation he took an interneship in a northern hospital. As a result of the climatic change it was reported that he had had very little trouble.

CASE V.—J. M. C. G. (Hospital number 206037), a white painter, aged sixty-six years, complained of attacks of coughing, expectoration of pus, acute shortness of breath, palpitation, pain over the heart and weakness. According to his story the symptoms had appeared only eight months previously, that is in May, 1936. Not until November, 1936 had they progressed to the point of severity necessitating medical attention. He had lost 30 pounds in weight. There was no history of idiosynerasy or allergy of any type and no history suggestive of previous tuberculosis in himself or in his family.

The peripheral arteries were generally sclerosed. The blood pressure was 130/70. The heart was negative. The pulmonary findings were those of asthmatic bronchitis. The sputum, however, contained a few tubercle bacilli along with myriads of other organisms. The roentgenologist reported extensive bilateral old pulmonary tuberculosis.

On Jan. 19, 1937 he had a severe attack of difficult breathing which was only partly relieved by an injection of 1 c.c. of adrenalin solution. On Jan. 20, 1937 during the night he developed an attack of respiratory distress which was refractory to adrenalin (1 c.c.) treatment but gradually let up after 1 c.c. or 0.24 gm. (3½ gr.) of aminophyllin intravenously. On Jan. 20, 1937 he had severe early morning attacks of dyspnea which were relieved by 0.48 gm. of aminophyllin (7½ gr.) intravenously, and Dr. Peticolas found that the vital capacity rose from 1,200 c.c. to 1,400 c.c. while the respiratory rate of 20 remained unchanged, the pulse dropped from 88 to 80 and the blood pressure remained 120/80. On Jan. 21, 1937 another attack responded to aminophyllin 0.48 gm. (7½ gr.) i.v. but the patient complained of a peculiar sensation over the precordium not a true pain. Its exact nature could not be determined though it persisted for several hours.

At 4 A.M. Feb. 18, 1937 he had another bad spell of coughing and struggling for breath. It passed off thirty minutes after an intravenous injection of 0.24 gm. (3½ gr.) of aminophyllin. On March 4 at about 4:30 A.M. he again became extremely dyspneic and an intravenous injection of 0.46 gm. of aminophyllin relieved him completely. On March 17 he suffered from a status asthmaticus with his vital capacity depressed to 800 c.c., a respiratory

TABLE II

EFFECT OF AMINOPHYLLINE (0.48 GM.) INTRAVENOUSLY IN STATUS ASTHMATICUS IN OUT PATIENTS

NAME, COLOR, AGE, SEX OUT-PATIENT NUMBER	DIAGNOSES	PREVIOUS MEDICINE (IN E. R., ETC.)	DURATION OF ASTHMA DURATION OF ATTACK	DOSE (OTHER THAN 0.48 GM.)	RELIEF, IMMEDIATE	INTERVAL UNTIL NEXT ATTACK	REACTION (IF ANY)	REMARKS
R. E. B.; W, 27, M. 67382	Br. asthma	Adren., ♏ x-xv Ephedrine, gr. ¾ Tr. Benz. Co. Morphine, gr. ¼ Atropine, gr. 1/150 Glucose I-V	11 Years 11-24 hours	gr. iii gr. iv	Complete Complete Complete	4 hours 2 months 4 months	None noted	Has not been to clinic with "Asthma" as chief complaint since Oct. 20, 1936, when he last received aminophylline. The adrenalin, etc., conspicuously failed to relieve him.
H. M.; B, 27, F. 16700	Br. asthma Syphilis, tertiary Cervicitis	Adren., ♏ x-xv Ephedrine, gr. ¾ Atropine, gr. 1/75 Ca. gluconate I-V	8 Years 2-12 hours		Complete Complete Complete	One year 6 days 4 days	"Hot flash" following injection on one occasion	Has been treated at least once by almost every interne for past three years. Other medicine afforded only irregular relief.
H. S.; B, 33, M. 9060	Br. asthma	Adren., ♏ x Ephedrine, gr. ¾	"Since childhood" 4 hours		Partial (delayed)	Two weeks One week	No reaction noted	Relieved on one occasion when two injections of adrenalin same night had failed. None-to-complete relief with adrenalin.
D. T. M.; B, 41, M. 75023	Br. asthma Syphilis, tertiary	Adren., ♏ x-xv Ephedrine, gr. ¾	1½ Years Minutes—hours		Partial	8 days	Some feeling of oppression over chest	Aminophylline given only one time. Succeeded after ♏ x adrenalin same day had failed.
J. B.; B, 44, M. 61141	Br. asthma	Adren., ♏ x-xv Ephed., gr. ⅜	20 Years		Complete	5 months	No reaction noted	Was treated with Aminophylline only one time, and on that occasion it succeeded after ♏ x and ♏ xv of adrenalin had failed.

TABLE II—CONT'D

NAME, COLOR, AGE, SEX OUT-PATIENT NUMBER	DIAGNOSES	PREVIOUS MEDICINE (IN E. R., ETC.)	DURATION OF ASTHMA DURATION OF ATTACK	DOSE (OTHER THAN 0.48 GM.)	RELIEF, IMMEDIATE	INTERVAL UNTIL NEXT ATTACK	REACTION (IF ANY)	REMARKS
J. B.; W, 51, M. 56101	Br. asthma	Adren., ♏ x	17 Years	gr. viiss gr. ii	Complete Complete	3 months 3 months	No reaction noted after either injection	Periodic attacks for seventeen years. Adrenalin usually relieved attacks during this period.
J. H.; B, 37, M. 58351	Br. asthma Urethral stricture	Adren., ♏ x-xv Ephedrine, gr. ¾ Amyl nitrite Morphine Amytal Autog. vaccine	9 Years Seasonal Minutes to 8 hours		Complete	One month	None noted	Rapid recovery from attack after administration of Aminophylline.
W. H. W.; B, 62, M. 13787	Chr. Br. asthma Hypertensive H. D.	Adren., ♏ x Ephedrine, gr. ¾	About 20 years 2-4-8 hours		Complete	One month	"Hot flash," very transient	Had only one dose of Aminophylline in 1935, that was given with ephedrine. Prompt relief usually followed adrenalin and ephedrine.
B. W.; B, 37, F. 14212	Asthma bronchitis Hay fever Gastro-intestinal allergy	Adren., ♏ x-xv Ephed., gr. ¾ Autog. vaccine Morph. sulf.. gr. ¼ Atropine gr. 1/150 Ca. gluconate I-V Adren., ♏ xii Amyl nitrite	12 Years 4-10 hours Not effective		Partial (delayed) Partial (delayed) Very Little	One day 24 days —?—	No reaction noted in any instance	Termed an "adrenalin-fast" case by several internes. All types of medications had failed signally in many of her visits to the emergency room and to the dispensary.

rate of 28, pulse rate of 100, and blood pressure of 128/86. After 0.48 gm. of aminophyllin had been given intravenously his vital capacity rose to 1,400 c.c. his respiration dropped to 24, and his pulse rate to 88, while his blood pressure rose to 142/84, and he was completely relieved. His vital capacity rose to 1,500 c.c. on March 21, and on March 27 it was 1,700 c.c.

CASE VI.—W. E. R. (Hospital number 46007), a male, cook, aged sixty-three, began to have asthmatic attacks in 1930 as a sequence of fall and winter colds with cough and chest pains which had been recurrent and of increasing severity for several years. Exertion would often bring on attacks of wheezing in the chest, choking sensations, vise-like pain in the left chest around the nipple radiating to the left shoulder, followed by a hacking cough with some white mucous expectoration. At times there was sputum. Vertigo usually came on at the end of an attack. Palpitation, weakness, loss of weight had occurred. The pain was vise-like in character brought on by exertion and by lying on the left chest and then arising. The patient had an occasional night sweat.

The chief physical abnormalities were found in the examination of the chest which was barrel shaped with a scar of an old liver drainage at the right base anteriorly and hyperesthesia about the left nipple. The chest was hyperresonant and showed the signs of asthmatic bronchitis. The heart was not enlarged. The blood pressure was normal. The heart rate was 68. The aortic second sound was slightly accentuated. No murmurs were heard. The peripheral vessels were moderately sclerosed. The blood pressure was 128/75 and 135/82. A roentgenogram showed signs of old fibroid pulmonary tuberculosis for the most part in the upper half of the right lung but to some extent throughout both lungs there were multiple pleurodiaphragmatic adhesions. The electrocardiogram showed slurring and low voltage in all leads, some left axis deviation but no pathognomic electrocardiographic signs were present.

On Oct. 12, 1934 his vital capacity was found to be 46.3 per cent of normal. On Feb. 7, 1935 his vital capacity was estimated to be 1,850 c.c. which for his 1.5 square meters body surface was 50 per cent of normal. The blood pressure dropped to as low as 105/65. He had been treated with codeine, novatropine, phenobarbital, ephedrine, atropine, and digitalis. He was maintained on the latter. The coughing spells continued. Tightness in the chest developed and dyspnea increased. Paroxysms of coughing which usually occurred in the early morning hours often developed into status asthmaticus and caused him to struggle for air. At such times piping high pitched musical râles were heard in both chests, particularly on inspiration. There seemed to be an acute vesicular emphysema engrafted upon a chronic emphysema. His vital capacity was reduced to 27 per cent of normal.

On Jan. 21, 1937 at 2:30 A.M. he had a sharp severe attack. His vital capacity was found to be only 700 c.c. with respiration 24, pulse 100, and blood pressure 110/70. He was given 0.48 gm. of aminophyllin intravenously in 10 c.c. of saline and his distress immediately subsided. His respirations dropped to 20 but his vital capacity, pulse, and blood pressure were unchanged in spite of striking symptomatic relief. He was, however, unable to go to sleep because of the excitation produced by the xanthine.

CASE VII.—B. C. (Hospital number 32683), a housewife, was admitted in status asthmaticus in which distressing condition she said she had been more or less constantly for two and one-half years, day and night, summer and winter. Her first attack occurred in 1920, when after recovering from the measles she had a smothering spell and wheezing in the chest. This attack cleared up shortly only to recur at first only after she caught cold or had upper respiratory infection to which she was particularly susceptible. Later sweeping and eating brought on the attack and finally they came on spontaneously. At first she was relieved by ephedrine and adrenalin but the drugs gradually became less and less effective and finally the relaxation lasted only about ten or fifteen minutes. She was worse after the effects wore off. She had lost 38 pounds in eight months before admission. She had been under the care of an allergist and had been tested with all sorts of pollens, emanations, dusts, and foods and was found to be only slightly sensitive to house dust but to nothing else. The patient had one brother who had had asthma but had recovered.

On physical examination she was orthopneic, unable to lie down, cyanotic and in severe distress. She had had frequent attacks of sinusitis and drainage of pus from the maxillary had been accomplished. The accessory muscles of respiration were constantly used and well developed. The chest was somewhat barrel shaped, emphysematous. Hyperresonance was noted on percussion. The signs of severe asthmatic state were present. Whistling, squeaking, sonorous groans and râles were heard on inspiration and on expiration. The heart was not enlarged. No murmurs were heard and no abnormal sounds. The blood pressure was 125/90. Scars, the result of numerous hypodermic injections were found on the extremities.

The roentgenologist reported extensive fibrosis in the right lung and, to a slightly less extent, the left. There was chronic bronchitis with an old pleuropneumonic disease of the right base. The left antrum and ethmoids were clouded.

The left antrum had been radically operated upon and upon subsequent puncture yielded a tablespoonful of mucopurulent exudate.

Records were unfortunately not kept of the early emergency treatment of this case. However, the progress note of Sept. 11, 1926 stated that 0.48 gm. (7½ gr.) of aminophyllin in 10 c.c. of saline intravenously had had a marked effect at the beginning of her hospitalization but had become less effective. Aminophyllin 0.1 gm. or 1-gr. doses t.i.d. p.o. was also tried in this case without any apparent benefit. Aminophyllin solution containing 0.6 gm. or 10 gr. per rectum was successful in one instance. Nevertheless, the patient was noted to have improved generally and was reported as sleeping better. The asthmatic attacks recurred but she had relatively long free periods such as she had not enjoyed for two or three years and adrenalin again became useful. There were few changes in her chest signs.

She was submitted to a course of typhoid vaccine intravenously which gave her a severe chill and temperatures that rose to 104° F. after which she felt better. After this adrenalin again became more effective as it did following the aminophyllin therapy. On Oct. 16, 1936 she was up and about the ward and while walking she became warm and suffered a most severe asthmatic attack. She, however, responded immediately to adrenalin. Because of the climatic conditions she was advised that she would probably continue her improvement more certainly in a higher drier climate and was therefore discharged home.

It is indeed unusual for a patient to become refractory to the effects of aminophyllin intravenously as this patient apparently did. Aminophyllin did not cause in her any more excitement or feeling of anxiety than adrenalin. It was effective longer, was not followed by a letdown, such as followed adrenalin, and seemed to restore the desirable reactivity to adrenalin.

SUMMARY AND CONCLUSIONS

Sixteen clinical cases of asthmatic bronchitis or chronic bronchial asthma presenting attacks of status asthmaticus are recorded, in which 41 injections of theophylline ethylene diamine (aminophyllin) had been administered intravenously as emergency therapy, usually with success after adrenalin had been ineffectual. Of these 41 injections 31 afforded prompt, complete, and persistent relief, but at least 6 of these were of the half dosage of 0.24 gm. or 3¾ gr.

In two instances the full dose of 0.48 gm. (7½ gr.) seemingly failed. There was one failure to accomplish relief and even in this instance posttheophylline e. d. adrenalin injections became effective. In one instance the patient was made slightly more uncomfortable.

In practically all cases that had become adrenalin fast theophylline e. d. seemed to restore the sensitiveness to adrenalin.

In only one instance was there the development of anything that might be considered a refractoriness to theophylline e. d.

Reactions to the intravenous injection of 0.48 gm. (7½ gr.) of theophylline e. d. were common but as a rule quite mild and innocuous and rarely very dis-

turbing. The precautions of diluting the 2 c.c. containing 0.48 gm. of theophylline e. d. to 10 c.c. with saline or glucose solution and injecting it slowly through a fine 22 gauge needle were always taken.

Theophylline e. d. in 0.48 gm. doses diluted to 10 c.c. and introduced slowly intravenously seems to be a most effective, prompt, reliable and safe therapeutic procedure for the combating of status asthmaticus even after there has developed a refractoriness to adrenalin.

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URINARY IRON EXCRETION†

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THE literature reveals a marked difference of opinion as to the amount of iron excreted in the urine (Table I). Some of these discrepancies may be accounted for by variations in the sensitivity or the technique of the method employed, but this can hardly account for the great variation in the results obtained by Lintzel and by Lanyar, Lieb, and Verdina who found no urinary iron, and that of Riecker who found 19.79 mg. per twenty-four hours in a case of pernicious anemia. The present investigation was undertaken because of this lack of agreement, and because no comparison had been made between the urinary iron output in men and in women.

*5 to 12 Quoted from Report of Council on Pharmacy and Chemistry. Therapeutic claims for theobromine and theophylline preparations, J. A. M. A., **94**: 1306, 1930.

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PROCEDURE

One hundred men and one hundred women from the Staff and from the Medical wards of the University Hospital served as subjects for this study, and none was included whose urine contained blood or either chemical or microscopic examination. The subjects were allowed to continue their ordinary routine as far as diet and medications were concerned, but patients receiving

TABLE I

URINARY IRON EXCRETION—REPORTS IN LITERATURE

AUTHOR	NO. OF CASES	AGE OF SUBJECTS	DIAGNOSIS	AVERAGE DAILY URINARY IRON EXCRETION MG.	REFERENCE
Bassett, S. H., Elden, C. A., and McCann, W. S.	2	Adult	Normal	Trace to less than 1 mg.	J. Nutrition **5**: 1, 1932
	1	Adult	Nephritis	0.6 mg.	
Daniels, A. L., and Wright, O. J.	8	4-6 yr.	Normal	0.22-0.55 mg.	J. Nutrition **8**: 125, 1934
Dry, T. J.	1	Adult	Hemochromatosis	0.18-0.69 mg.	Minnesota Med. **17**: 301, 1934
Farrar, G. E., and Goldhamer, S. M.	4	Adult	Normal	0.02-0.03 mg. per 100 c.c.	J. Nutrition **10**: 241, 1935
Hanzal, R. F., and Bing, F. C.	3	Adult	Normal	0.13-0.42 mg.	Proc. Soc. Exper. Biol. & Med. **31**: 617, 1934
Henriques, V., and Roland, H.	4	Adult	Normal	0.09-0.19 mg.	Biochem. Ztschr. **201**: 479, 1928
	21	Adult	Diabetes mellitus	0.08-0.32 mg.	
	5	Adult	Peptic ulcer	0.08-0.19 mg.	
	2	Adult	Anemia	0.10-0.13 mg.	
Lanyar, F., Lieb, H., and Verdino, A.	3	Adult	Diabetes mellitus	0	Ztschr. f. physiol. Chem. **217**: 160, 1933
	5	Adult	Polycythemia vera	0	
	1	Adult	Leukemia	Less than 0.005 mg.	
	4	Adult	Pernicious anemia	0 to trace	
	5	Adult	Hemolytic icterus	0	
Leichsenring, J., and Flor, I. H.	4	36-56 mo.	Normal	0.11-0.81 mg.	J. Nutrition **5**: 141, 1932
Lintzel, W.	3	Adult	Normal	0	Ztschr. f. Biol. **89**: 350, 1929
Marlow, A., and Taylor, F. H. L.	2	Adult	Normal	0.06-0.10 mg.	Arch. Int. Med. **53**: 551, 1934
	3	Adult	Hypochromic anemia	0.02-0.05 mg.	
	2	Adult	Pernicious anemia	0.037-0.59 mg.	
McClure, C. W.	1	Adult	Hemochromatosis	0	Arch. Int. Med. **22**: 610, 1918
Riecker, H. H.	6	Adult	Pernicious anemia	1.99-19.79 mg.	Arch. Int. Med. **46**: 458, 1930
Rose, M. S., Vahlteich, E., Robb, E., and Bloomfield, E. M.	1	2½ yr.	Normal	0.16-0.22 mg.	J. Nutrition **3**: 229, 1930
Sherman, H. C.	1	Adult	Normal	0.2 mg.	U. S. Dept. Agr. Office Exper. Station Bulletin 185, 1907
Stockman, R., and Greig, E. D. W.	3	Adult	Normal	0.66-1.26 mg.	J. Physiol. **21**: 55, 1897
Vahlteich, E., Funnell, E. H., Macleod, G., and Rose, M. S.	2	Adult	Normal	0.10-0.40 mg.	J. Am. Dietet. A. **11**: 331, 1935

TABLE II

URINARY IRON LOSS—MALES

CASE	DIAGNOSIS	AGE	BLOOD		DAILY IRON INTAKE MG.	AVERAGE DAILY URINE		
			HEMATOCRIT %	HEMOGLOBIN GM.		VOL. C.C.	CREATININE GM.	IRON MG.
1	Obesity	41	46.0	13.750	10.79	628	2.456	0.09
2	Hypertrophic arthritis	66	38.0	11.570	13.74	413	1.115	0.10
3	Malignant lymphoma	37	36.0	12.410	14.83	403	1.260	0.10
4	Rheumatic heart disease	51	44.0	12.625	11.43	743	1.492	0.11
5	Neurofibromatosis	55	45.0	12.625	14.83	790	1.193	0.12
6	Carcinoma of stomach	63	33.5	9.620	6.78	457	0.993	0.13
7	Coronary arteriosclerosis	53	42.0	11.990	11.53	687	1.617	0.14
8	Infectious arthritis	55	45.0	13.470	14.83	1447	1.627	0.14
9	Amyotrophic lateral sclerosis	68	42.5	12.200	14.83	1500	1.180	0.15
10	Carcinoma of stomach	48	23.0	6.140	12.25	800	1.493	0.16
11	Hypertrophic arthritis	74	37.0	10.220	14.83	703	1.087	0.18
12	Bronchial asthma	20	44.0	12.200	14.83	580	1.561	0.18
13	Epilepsy	17	47.0	12.910	12.88	730	1.989	0.18
14	Carcinoma of stomach	51	17.0	4.210	12.88	740	1.097	0.19
15	Peptic ulcer	70	34.0	9.920	14.61	1093	1.399	0.19
16	Infectious arthritis	29	40.5	11.570	14.83	753	1.555	0.19
17	Infectious arthritis	33	39.0	10.520	14.83	787	1.342	0.20
18	Hemorrhoids	25	40.0	11.360	11.43	1023	1.281	0.20
19	Psychoneurosis	61	38.5	11.990	14.83	2017	2.138	0.20
20	Infectious arthritis	47	48.0	12.625	14.83	1300	1.393	0.20
21	Peripheral neuritis	58	48.0	14.380	14.83	993	1.714	0.20
22	Infectious arthritis	63	37.0	9.320	21.82	683	1.380	0.21
23	Neurosyphilis	67	40.5	11.150	13.74	683	1.033	0.21
24	Arthritis of spine	37	47.0	12.625	14.83	846	1.255	0.21
25	Infectious arthritis	50	41.0	11.150	12.25	1093	1.061	0.22
26	Peptic ulcer	46	42.0	11.990	14.83	1140	1.664	0.23
27	Diabetes mellitus	68	36.0	11.150	13.44	1623	1.434	0.24
28	Pulmonary tuberculosis	60	40.0	10.220	12.88	1007	1.083	0.25
29	Inguinal hernia	33	34.0	10.940	14.83	1746	1.867	0.26
30	Syphilitic aortitis	60	43.5	11.780	11.53	877	1.512	0.26
31	Emphysema	43	43.0	12.200	11.43	1033	1.494	0.26
32	Arteriosclerosis	53	39.5	12.625	14.83	1020	1.381	0.26
33	Bronchial asthma	40	51.0	13.750	14.83	1320	1.374	0.26
34	Ulcerative colitis	35	30.0	7.100	11.43	1070	1.641	0.27
35	Rheumatic fever	21	37.0	11.990	14.83	627	1.045	0.28
36	Diabetes mellitus	51	40.0	12.910	13.43	1417	0.922	0.28
37	Spastic colon	18	47.0	14.030	14.83	927	2.180	0.28
38	Carcinoma of bronchus	67	35.0	8.680	15.92	733	1.292	0.29
39	Nephrosclerosis	60	29.0	9.170	11.53	1150	1.724	0.29
40	Infectious arthritis	31	44.5	12.910	14.83	1170	1.559	0.29
41	Epilepsy	21	46.0	13.750	14.83	740	1.887	0.30
42	Rheumatic heart disease	31	43.0	12.410	14.83	1053	2.086	0.31
43	Psychoneurosis	35	42.0	11.150	14.83	1053	1.076	0.32
44	Pulmonary tuberculosis	47	45.0	11.570	14.83	1293	1.892	0.32
45	Carcinoma of pancreas	50	42.5	13.190	16.04	1607	1.678	0.32
46	Infectious arthritis	65	38.0	9.470	14.83	950	1.353	0.33
47	Hemochromatosis	53	45.0	11.100	13.42	1113	1.154	0.33
48	Psychoneurosis	33	42.0	12.200	12.88	1640	2.037	0.33
49	Tumor cerebri	17	45.5	12.410	14.83	813	2.075	0.33
50	Coronary arteriosclerosis	50	40.5	12.410	11.53	1313	1.451	0.33
51	Epilepsy	25	43.0	10.220	14.83	2330	2.155	0.35
52	Chronic constipation	31	48.0	12.410	12.25	1410	1.784	0.35
53	Carcinoma of colon	53	22.0	5.795	11.51	890	1.430	0.36
54	Sciatica	43	35.0	9.620	14.83	1823	2.325	0.36
55	Pernicious anemia	58	38.0	11.990	14.83	893	1.430	0.36
56	Bronchiectasis	34	41.0	10.520	14.83	1057	1.555	0.37
57	Lead poisoning	32	28.0	8.150	12.75	912	1.402	0.38
58	Peptic ulcer	23	41.0	12.625	12.25	1903	1.643	0.38
59	Bronchiectasis	45	50.0	13.750	14.83	1267	2.201	0.38
60	Postural hypotension	63	30.0	7.715	20.74	1567	1.131	0.39

TABLE II—CONT'D

CASE	DIAGNOSIS	AGE	BLOOD		DAILY IRON INTAKE MG.	AVERAGE DAILY URINE		
			HEMATOCRIT %	HEMOGLOBIN GM.		VOL. C.C.	CREATININE GM.	IRON MG.
61	Carcinoma of lung	27	33.0	9.020	12.88	970	1.683	0.39
62	Peptic ulcer	36	42.5	11.570	12.25	1563	1.522	0.39
63	Peptic ulcer	49	46.0	13.750	12.25	1943	1.876	0.39
64	Pernicious anemia	60	22.0	5.740	14.83	790	1.258	0.40
65	Coronary arteriosclerosis	78	39.0	12.200	13.74	993	1.278	0.40
66	Arterial hypertension	24	43.5	12.625	14.83	2633	2.755	0.40
67	Spastic colon	53	44.0	12.625	11.43	1093	1.769	0.44
68	Hypertrophic arthritis	51	42.0	12.200	14.83	1030	1.514	0.46
69	Syphilitic aortitis	62	39.0	12.200	12.25	1857	1.581	0.46
70	Peptic ulcer	58	41.0	12.625	12.25	1840	1.469	0.46
71	Glomerular nephritis	26	40.0	12.625	14.83	1357	1.880	0.47
72	Psychoneurosis	32	41.0	13.190	14.83	2357	1.950	0.47
73	Infectious arthritis	55	39.0	10.940	12.88	1380	1.418	0.48
74	Hemochromatosis	48	44.0	11.360	13.43	1633	0.933	0.49
75	Nephrosclerosis	55	31.0	8.570	11.53	1853	1.215	0.50
76	Peptic ulcer	51	43.0	12.625	11.43	1013	1.162	0.51
77	Hypertrophic arthritis	47	44.0	11.570	14.83	1017	1.999	0.53
78	Myxoedema	59	39.0	12.420	12.19	1330	1.220	0.53
79	Tumor cauda equina	43	47.0	14.380	14.83	2110	1.843	0.53
80	Infectious arthritis	27	34.5	10.940	14.83	1340	1.234	0.54
81	Neurosyphilis	27	46.0	11.990	14.83	1787	1.790	0.54
82	Arteriosclerosis	59	36.0	10.070	14.83	1933	1.976	0.58
83	Hyperthyroidism	50	43.0	13.190	14.83	1503	1.337	0.60
84	Peptic ulcer	51	44.0	13.190	12.25	2447	1.961	0.61
85	Infectious arthritis	35	37.0	10.940	14.83	1580	1.712	0.63
86	Hemochromatosis	50	41.0	12.200	12.90	1580	1.386	0.63
87	Peptic ulcer	57	44.0	10.370	11.43	2135	1.099	0.64
88	Peptic ulcer	74	35.0	10.070	2.46	1627	1.489	0.65
89	Lung abscess	47	38.0	10.220	12.88	2060	1.746	0.65
90	Refractive error	35	37.0	11.150	14.83	1737	1.347	0.69
91	Psychoneurosis	40	42.0	12.625	11.43	2323	1.832	0.70
92	Neurosyphilis	39	48.0	14.380	14.83	2087	1.878	0.73
93	Peptic ulcer	47	5.5	1.590	7.18	1183	1.314	0.77
94	Thromboplebitis	38	45.0	12.410	12.25	2203	1.769	0.77
95	Malignant lymphoma	30	42.0	11.570	14.83	2520	2.149	0.82
96	Bronchial asthma	46	43.0	11.780	12.88	2047	2.048	0.82
97	Polycythemia vera	48	51.0	19.740	12.33	1558	1.456	0.90
98	Spastic colon	29	45.0	13.190	12.25	3390	2.010	1.01
99	Bronchiectasis	38	46.0	12.625	14.83	2337	1.370	1.09
100	Infectious arthritis	22	42.0	11.570	12.88	2863	1.978	1.29

Average daily urinary iron excretion 0.395 mg.

supplementary iron or excessive fluids were not included. All analyses on women were made during the intermenstrual period. The iron content of the diet was calculated from the tables of Waller,[1] and was used as the approximate daily iron intake.

Complete urine specimens for three days were used as a basis for the study, the urine being collected directly in glass containers and proper precautions were taken to prevent iron contamination. The creatinine excretion was determined for each day to make sure that collection of the specimen had been complete. Triplicate iron determinations were made on aliquots of the pooled three-day specimens according to an adaptation of the method of Reis and Chakmakjian.[2]

An hematocrit reading was made on each patient by the Van Allen method,[3] and the hemoglobin was determined by the Newcomer method.[4]

TABLE III

URINARY IRON LOSS—FEMALES

CASE	DIAGNOSIS	AGE	BLOOD		DAILY IRON INTAKE MG.	AVERAGE DAILY URINE		
			HEMATOCRIT %	HEMOGLOBIN GM.		VOL. C.C.	CREATININE GM.	IRON MG.
1	Peptic ulcer	42	37.0	10.370	11.43	273	1.192	0.08
2	Cholelithiasis	33	31.0	9.770	12.88	460	0.924	0.09
3	Infectious arthritis	24	41.0	12.410	14.83	683	1.120	0.10
4	Pernicious anemia	41	32.0	8.795	14.83	810	1.047	0.12
5	Spastic colon	39	33.5	8.905	11.43	637	0.960	0.13
6	Psychoneurosis	18	37.5	11.150	14.83	410	0.911	0.14
7	Psychoneurosis	39	35.0	9.920	11.43	607	1.541	0.15
8	Chronic constipation	70	37.0	9.920	12.88	1075	0.806	0.16
9	Hypertrophic arthritis	60	44.5	11.480	14.83	900	1.043	0.16
10	Peptic ulcer	59	41.0	12.625	11.53	633	0.854	0.16
11	Congenital syphilis	38	30.0	8.570	12.88	935	1.068	0.19
12	Ulcerative colitis	46	42.5	11.780	11.43	753	1.041	0.21
13	Hypertrophic arthritis	36	40.0	13.190	12.88	833	1.548	0.21
14	Rheumatic heart disease	34	36.0	10.520	14.83	1450	1.342	0.22
15	Bronchial asthma	30	40.0	12.200	9.81	897	0.847	0.22
16	Pneumonia	38	32.5	9.470	11.51	1150	0.836	0.23
17	Malignant lymphoma	33	25.0	6.085	20.74	800	0.987	0.24
18	Normal	31	39.0	12.200	14.83	1175	1.555	0.24
19	Glomerular nephritis chronic	20	43.0	12.200	14.83	1003	1.374	0.25
20	Spastic colon	21	37.0	10.220	11.43	1700	1.113	0.26
21	Rheumatic heart disease	54	36.5	11.480	11.43	1040	0.905	0.26
22	Psychoneurosis	44	40.0	11.570	14.83	1737	1.334	0.26
23	Neurosyphilis	27	42.0	11.990	14.83	1033	0.770	0.26
24	Coronary arteriosclerosis	38	39.0	11.990	11.53	1020	1.007	0.26
25	Idiopathic hypochromic anemia	34	24.5	5.595	12.20	685	0.965	0.27
26	Hemorrhoids	43	27.0	7.015	11.89	1330	1.108	0.27
27	Psychoneurosis	56	40.0	12.200	12.88	1820	1.339	0.27
28	Menorrhagia	46	31.0	7.715	11.89	1102	1.216	0.28
29	Normal	24	43.0	9.920	14.83	568	1.234	0.28
30	Menorrhagia	44	26.0	7.450	10.17	1430	1.394	0.29
31	Normal	21	35.0	8.905	14.83	970	1.278	0.29
32	Normal	31	37.0	11.000	14.83	825	–	0.29
33	Adenoma of thyroid	43	37.5	11.570	16.04	1153	0.952	0.29
34	Idiopathic hypochromic anemia	43	17.5	4.075	12.22	1003	1.079	0.30
35	Erythema multiforme	33	35.0	10.520	14.83	590	0.831	0.30
36	Normal	24	39.0	10.710	14.83	1515	1.364	0.30
37	Bronchiectasis	48	34.0	8.455	11.96	638	0.645	0.32
38	Migraine	31	34.0	8.680	14.83	643	1.138	0.32
39	Spastic colon	32	37.0	9.920	11.43	1613	1.275	0.32
40	Normal	43	44.0	12.625	14.83	1080	1.169	0.32
41	Psychoneurosis	57	43.0	12.910	16.04	1617	1.453	0.32
42	Hemorrhoids	52	18.0	5.645	11.86	2215	1.088	0.33
43	Lead poisoning	55	28.0	7.890	11.49	1138	0.798	0.34
44	Syphilis	37	36.0	10.220	14.83	850	–	0.34
45	Bronchial asthma	58	46.0	12.625	11.43	2253	1.161	0.34
46	Pulmonary infarct	20	29.5	8.005	12.88	1763	0.889	0.35
47	Pernicious anemia	53	38.5	8.905	14.83	1225	0.655	0.37
48	Psychoneurosis	31	36.0	10.370	14.83	1220	1.348	0.37
49	Erythema multiforme	29	37.0	8.455	14.83	1897	1.244	0.38
50	Arteriosclerosis	69	30.0	8.115	11.78	1290	0.992	0.39
51	Hyperthyroidism	36	39.0	11.370	20.74	967	1.126	0.39
52	Rheumatic heart disease	37	40.0	11.990	11.53	1030	–	0.39
53	Psychoneurosis	31	36.0	10.520	14.83	2053	1.316	0.41
54	Normal	36	37.0	12.510	14.83	1020	1.464	0.41
55	Normal	25	43.0	11.480	14.83	1400	1.278	0.42
56	Rheumatic heart disease	43	43.5	10.070	11.53	1510	1.196	0.45
57	Rheumatic heart disease	33	33.5	10.940	11.53	1783	1.783	0.45
58	Idiopathic hypochromic anemia	40	34.0	8.570	14.83	1523	1.420	0.46
59	Rheumatic heart disease	29	48.0	13.190	11.53	11.93	0.934	0.46
60	Myxoedema	53	27.0	7.015	11.91	2358	0.915	0.47

TABLE III—CONT'D

CASE	DIAGNOSIS	AGE	BLOOD		DAILY IRON INTAKE MG.	AVERAGE DAILY URINE		
			HEMATOCRIT %	HEMOGLOBIN GM.		VOL. C.C.	CREATININE GM.	IRON MG.
61	Hyperthyroidism	25	43.0	10.220	20.74	1573	1.323	0.47
62	Hypochromic anemia	46	29.0	8.100	9.13	1077	0.854	0.47
63	Infectious arthritis	41	41.0	11.990	11.78	953	1.176	0.47
64	Normal	31	40.0	11.940	14.83	1235	1.315	0.49
65	Idiopathic hypochromic anemia	40	22.0	4.785	11.89	1658	1.265	0.50
66	Pernicious anemia	38	36.0	11.150	14.83	1807	0.916	0.54
67	Menorrhagia	46	31.0	7.715	11.89	1818	0.927	0.55
68	Obesity	56	43.0	12.625	14.83	1383	1.363	0.55
69	Normal	38	38.0	10.030	14.83	1850	1.284	0.56
70	Hypertrophic arthritis	61	42.0	13.190	11.53	980	0.922	0.57
71	Psychoneurosis	38	40.0	12.625	12.88	2400	1.329	0.60
72	Idiopathic hypochromic anemia	35	23.0	5.740	10.31	1918	0.893	0.65
73	Idiopathic hypochromic anemia	40	30.5	7.450	11.89	1697	0.758	0.67
74	Idiopathic hypochromic anemia	37	26.0	5.500	10.26	1437	0.830	0.68
75	Peptic ulcer	53	39.0	12.625	11.81	995	0.933	0.70
76	Idiopathic hypochromic anemia	34	31.0	6.140	12.67	2410	0.998	0.72
77	Menorrhagia	23	31.5	5.970	13.03	2428	1.375	0.73
78	Lobar pneumonia	44	42.0	10.370	12.88	2910	1.217	0.73
79	Psychoneurosis	38	39.0	10.520	20.74	2073	2.590	0.73
80	Menorrhagia	34	28.0	6.255	11.96	1073	1.100	0.75
81	Malnutrition	39	39.5	10.200	14.83	2155	–	0.75
82	Infectious arthritis	51	39.5	12.410	11.78	1863	1.358	0.75
83	Hypochromic anemia-pregnancy	39	26.0	7.710	9.96	1253	1.122	0.81
84	Diabetes mellitus	18	38.0	10.810	12.89	1177	0.908	0.82
85	Idiopathic hypochromic anemia	41	41.0	12.510	13.14	1435	0.998	0.84
86	Menorrhagia	23	28.0	7.450	12.59	2159	1.058	0.86
87	Bronchial asthma	22	41.0	12.410	12.88	2173	1.393	0.87
88	Menorrhagia	21	30.0	7.890	12.69	1315	0.856	0.88
89	Idiopathic hypochromic anemia	27	30.0	6.665	12.25	3029	1.084	0.91
90	Menorrhagia	50	31.0	10.200	10.12	1780	1.104	0.91
91	Hypochromic anemia	33	17.0	3.610	12.96	2315	1.715	0.93
92	Idiopathic hypochromic anemia	25	39.0	10.710	9.69	1610	0.933	0.98
93	Idiopathic hypochromic anemia	37	29.0	5.855	12.57	2467	0.854	0.99
94	Idiopathic hypochromic anemia	29	26.5	5.550	11.88	925	1.072	1.03
95	Idiopathic hypochromic anemia	47	28.0	7.890	10.33	1602	0.781	1.09
96	Hyperthyroidism	26	36.0	10.070	20.74	3120	1.721	1.09
97	Peptic ulcer	48	21.5	4.300	11.96	2200	0.915	1.10
98	Neurosyphilis	44	27.5	6.140	12.01	2765	1.250	1.11
99	Menorrhagia	26	20.0	4.360	9.98	1588	1.039	1.23
100	Diabetes mellitus	19	42.0	12.050	12.85	903	0.950	1.63

Average daily urinary iron excretion 0.489 mg.

RESULTS

The results of this study of urinary iron excretion are given in Tables II and III. It is to be noted that there is no correlation between the age of the subjects (eighteen to seventy-eight years), and the iron excreted in the urine, nor between the creatinine excretion and the urinary iron loss.

It is apparent that some of the subjects excreting a larger volume of urine excreted slightly larger amounts of urinary iron, but this tendency is manifested in too small a percentage of cases to be of significance so that no correlation between the iron excretion and the urinary volume, as has been suggested by Farrar and Goldhamer,[5] could be detected.

The dietary iron intake of these subjects varied from 2.46 to 21.82 mg. per day, and there was no apparent relationship between the urinary iron excretion and the iron intake within these limits. These results are in accord

with those of Coons,[6] who found that the amount of urinary iron was not related to the volume of urine nor was it proportional to the iron content of the diet. As a further indication that the urinary iron excretion for each individual is quite constant regardless of the iron intake, ten of the subjects were given medicinal iron in the form of iron and ammonium citrates, three grams per day. It will be seen from Table IV that this supplementary iron did not appreciably alter their urinary iron excretion. In another group of ten subjects the urinary iron excretion was determined on a second occasion after

TABLE IV

EFFECT OF IRON INTAKE ON URINARY IRON EXCRETION

CASE	DAILY IRON INGESTION MG.	AVERAGE DAILY URINE		
		VOLUME C.C.	CREATININE GM.	IRON EXCRETION MG.
1	12.2	1193	0.854	0.30
	518.1	1603	0.954	0.32
2	11.6	2062	1.350	0.41
	517.7	1780	1.280	0.36
3	11.9	1658	1.265	0.50
	517.7	2658	1.489	0.53
4	9.7	1196	0.838	0.64
	516.2	1919	0.893	0.65
5	12.5	1488	1.130	0.67
	518.9	612	0.941	0.67
6	10.0	1437	0.863	0.68
	515.4	1450	0.914	0.59
7	11.8	995	0.933	0.70
	517.6	1400	1.089	0.70
8	12.8	2410	0.998	0.72
	519.4	2613	1.053	0.78
9	12.0	2260	0.936	0.79
	517.3	2142	0.966	0.86
10	10.0	1588	1.039	1.23
	516.1	1853	1.098	1.23

TABLE V

URINARY IRON RETENTION
REPEATED OBSERVATIONS IN THE SAME INDIVIDUALS

CASE	DATE	AVERAGE DAILY URINE		
		VOLUME C.C.	CREATININE GM.	IRON EXCRETION MG.
1	4/20/35	1863	1.358	0.75
	5/23/35	1912	1.195	0.76
2	1/ 8/35	1003	1.079	0.30
	2/10/35	1603	0.954	0.32
3	3/31/36	1998	1.177	0.38
	5/ 9/36	1990	1.198	0.40
4	3/25/36	1523	1.420	0.46
	5/15/36	1780	1.280	0.36
5	12/ 5/34	1430	1.394	0.29
	2/ 4/35	1728	1.257	0.35
6	11/13/32	1183	1.314	0.77
	1/12/33	1697	1.308	0.86
7	12/ 4/35	1138	0.798	0.34
	2/13/36	1235	0.735	0.37
8	10/11/34	1658	1.265	0.50
	2/11/35	2658	1.489	0.53
9	10/20/34	1915	1.074	0.48
	3/ 2/35	1295	1.308	0.52
10	10/24/33	1435	0.998	0.84
	6/16/34	2048	0.970	0.82

an interval of from 30 to 235 days. It will be noted in Table V that the greatest variation between the two determinations in any patient was 0.1 mg. per day (Case 4). These results indicate that the urinary iron excretion, while varying from one case to another, is fairly constant for each individual.

The hemoglobin in these subjects varied from 1.590 to 19.740 gm. per 100 c.c. of blood, but no correlation could be found between the hemoglobin level and the amount of iron excreted in the urine. The range of urinary iron loss is slightly greater in women than in men, the average urinary iron in women being 0.489 mg. per day, while in men it was 0.395 mg., a difference of 24 per cent. The reason for this is not apparent and, since little is known of the metabolic processes which have preceded the excretion of iron in the urine, it is unwise to venture an explanation. When Tables II and III are examined further, it is seen that the average iron excretion in the 14 patients with idiopathic hypochromic anemia was 0.720 mg. per day, or 47 per cent more than the average urinary iron loss for the one hundred women, and 63 per cent more than the average for both men and women. The importance of this greater urinary iron loss in women is questionable, but the fact that it is still larger in cases of idiopathic hypochromic anemia may add to its significance. We do not believe this to be the cause of this type of anemia, but it is possible that it may be a contributing factor in certain cases.

SUMMARY

The urinary iron excretion of 100 men ranged from 0.09 to 1.29 mg. per day with an average of 0.395 mg.

The urinary iron excretion of 100 women during the intermenstrual period varied from 0.08 to 1.63 mg. per day with an average of 0.489 mg. This is 24 per cent greater than the urinary iron output in men.

There was no apparent correlation between the urinary iron and the age of the individual or the blood hemoglobin.

There was no constant correlation between the volume of the urine and the urinary iron.

Patients with idiopathic hypochromic anemia excreted a larger amount of urinary iron than any other group of patients studied.

The amount of iron excreted in the urine was fairly constant in the same individual regardless of the iron intake.

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USE OF MERCURIAL DIURETICS*

Case Report with Autopsy Findings

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DIURETICS are a recognized part of the management of edema due to congestive heart failure. Inorganic salts, xanthine compounds and organic mercurial preparations have their place. Christian[1] has given the indications for their usage. Keith[2] has recently summarized the actions of various diuretics. Novasurol, salyrgan and mercupurin in the order named are the mercurial preparations that have been introduced for intramuscular or intravenous use. Lately mercurit has been developed for rectal administration.

Mercupurin, introduced as Novurit, is a combination containing mercury and the purine compound theophylline. Although the first publication by Hahn[3] about it appeared in 1929 the first reference in American literature is that of Crawford and McDaniel.[4] There have been only a few reports about it in this country. Keith (loc. cit.) mentions it only in passing. Steuer and Wolpaw[5] report its use in 22 patients.

The patient whose clinical and postmortem findings make up this report was first seen Aug. 7, 1933. A minister, aged sixty-eight, complained of weakness, difficulty in breathing and swelling of the ankles. These complaints followed an acute upper respiratory infection in May, 1933. He had lost 21 pounds since the respiratory infection. The positive findings in examination were a markedly enlarged heart, blood pressure 184/120, slight dullness in both bases posteriorly with moist râles, enlarged liver, edema of genitals, legs, and ankles. Urinalysis showed a trace of albumin, one-plus hyalin and granular casts. Blood count was normal, blood Kahn negative. Phenosulphonephthalein output was 45 per cent in two hours. Blood chemical examinations read urea 48 mg., creatinine 1.5 mg., and sugar 115 mg., respectively, per 100 c.c. Gastrointestinal x-ray examination report read "no definite evidence of pathologic changes." Cholecystogram showed no dye entering the gallbladder. Shadow of a stone was seen.

Bed rest and digitalis relieved the signs of congestive failure. Edema disappeared. The patient was very anxious to resume his pastoral duties so after a month his condition was deemed good enough to permit this. He was able to carry on though with some effort and some dyspnea, particularly with preaching, until May, 1934. An attack of bronchopneumonia then necessitated giving up active work, though he was able to serve as supply minister until retirement in the fall of 1934. Digitalis was the only medication used during this period.

*From the Norbury Sanatorium.
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Urine output lessened and edema became more persistent in the fall of 1934. Caffeine, theobromine, theamin, theocalcin, and theophylline were used in turn. Each of these relieved dyspnea and produced moderate diuresis. This is in accord with the findings of Friedman, Resnik, Calhoun and Harrison[6] that diuretic drugs should be used frequently for ambulatory patients and not reserved for advanced cases when dropsy is established. The xanthine products were not sufficiently active to control the edema entirely with the lessening myocardial power. Consequently ammonium chloride was started on the fifth and salyrgan given on the eighth of November, 1934. The response in this case was excellent. Blood urea determination made at the time of the first injection was 66 mg. and at the second injection two weeks later 38 mg. per 100 c.c.

Salyrgan was given as needed for four months at gradually shortened intervals. Signs of portal obstruction increased so that while salyrgan took care of edema of extremities, ascites became distressing. Abdominal paracentesis was first performed March 4, 1935. This procedure with injection of salyrgan preceded a few days by administration of ammonium chloride, together with digitalis daily constituted the management until Aug. 31, 1935. Mercupurin was then available and was used until Sept. 26, 1936.

This patient was ambulatory and quite comfortable most of the time until the spring of 1936. In April he had an attack of coronary thrombosis which limited his activities thereafter although he was able to go out riding, to walk about the house, to visit with his friends until two weeks before his death Oct. 6, 1936. Death occurred from progressive myocardial failure of the congestive type.

Thirty-two injections of salyrgan and 58 injections of mercupurin were given this patient. Abdominal paracentesis was carried out 75 times. Kidney action was checked by weekly urinalysis and by urea determination on every lot of ascitic fluid. The urine did not show signs of renal damage by the mercurials used although cast counts were not done. Only once did ascitic fluid urea reach more than 50 mg. per 100 c.c. when it registered 55 mg. per 100 c.c. June 29, 1935. One month before death the reading was 42 mg. The average was 37.5 mg. The average twenty-four-hour urine output after either salyrgan or mercupurin was 4,500 c.c. The average amount of ascitic fluid secured with paracentesis was 3,520 c.c. The usual procedure was to start paracentesis and then inject the diuretic. The fluid loss in the subsequent twenty-four-hour period therefore averaged 8,020 c.c. Adequate fluid intake, moderately high protein and high ash diet were used to help maintain bodily fluid and body protein and to help make up mineral loss. At no time were there signs of the syndrome described by Poll and Stern.[7] Except for a short period of hospitalization when first under observation this patient was cared for at his home. He and his family were most cooperative in carrying out procedures and in observing effects of various measures used. His interest was such that several months before the end he and his family gave permission for autopsy at his death to facilitate further study.

A summary of the autopsy protocol is as follows: There was a moderate amount of chronic adhesive peritonitis as a result of the repeated aspirations. The omentum was adherent to the anterior abdominal wall in several places.

Fluid was present to the amount of 2,500 c.c. in the peritoneal, 250 c.c. in the pericardial, and 1,500 c.c. in the right pleural cavity. The heart showed hypertrophy and dilatation with some friability of the left ventricular wall on the anterior aspect. The mitral ring was dilated. There was chronic passive congestion of the lungs and spleen. The liver was enlarged with moderate hobnailed surface. Fibrous bands were seen running through the liver. Chronic passive congestion was present. The gallbladder wall was thickened. The gallbladder contained one large stone. The kidneys were normal in size and appearance except for congestion. The capsule stripped without much difficulty. Cut section showed normal cortical band with glomeruli standing out quite well. The gastrointestinal tract was not opened. It did not appear unusual except for adhesive peritonitis uniting some loops of intestine.

The autopsy findings were of interest in that they showed that repeated injections of both salyrgan and mercupurin did not show gross evidence of kidney injury in this case. The kidneys looked grossly better than was expected in an individual of seventy-one with hypertension. Wiseman[8] reported a case where 270 injections of salyrgan had been given over a period of five years. Smith[9] reported a case where 190 injections had been administered over a three-year period. Study of available literature has not shown any cases where as many doses of mercupurin have been given as reported here. Reports such as these indicate that in properly selected situations these drugs can be used without harmful effects. Patients with progressive myocardial weakness can be kept more active and more comfortable. Although the underlying organic condition renders cure impossible something can be done through management and medication to add to their time of usefulness and to give them more time with their families.

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THE EFFECT OF STAPHYLOCOCCUS ANTITOXIN ON RABBITS GIVEN BROTH CULTURES OF STAPHYLOCOCCI INTRAVENOUSLY*

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STAPHYLOCOCCUS antitoxin neutralizes the hemolytic, skin necrotizing and lethal action of staphylococcus toxin in vitro and in vivo.[1, 2] A single antitoxic serum inhibits the action of the toxin produced by different strains of staphylococci.[3] It has not been proved that staphylococci produced an exotoxin in vivo. Some experiments, however, show that rabbits inoculated with cultures of virulent staphylococci and given staphylococcus antitoxin live for a longer time than rabbits receiving only staphylococci.[4, 5] The survival of these passively immunized animals suggests that staphylococci do produce a toxin in vivo and that this toxin is neutralized by staphylococcus antitoxin. Bacteria are also phagocytosed more easily in the presence of immune serums than they are in the presence of normal serums.[6]

The present study was made to determine the effect of staphylococcus antitoxin on rabbits inoculated intravenously with an infusion broth culture of a virulent, toxin producing strain of staphylococci.

MATERIAL AND METHODS

A group of 31 normal rabbits was used. The organism is a hemolytic *Staphylococcus aureus* derived from a daughter colony of a hemolytic *Staphylococcus aureus* isolated from the pharynx of a patient with agranulocytic angina. This organism produces a hemotoxin and a skin necrotizing toxin when grown for forty-eight hours in veal infusion broth. The bacteria were grown in infusion broth for eighteen to twenty-four hours immediately preceding the injection into the animals. The amount of the inoculum varied in the different experiments; however the experimental and control groups in each experiment were always given the same quantity of the culture.

A part of the infusion broth culture of staphylococci used in Experiment 4 was centrifuged and the supernatant was titrated for hemotoxin and a skin necrotizing toxin.

The staphylococcus antitoxin† contained approximately 350 units per cubic centimeter. A 5.0 c.c. volume of this antitoxin was given at each injection. Both the antitoxin and the staphylococci were inoculated intravenously in the marginal ear veins.

The animals in the control group were given only the infusion broth cultures of staphylococci.

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†The antitoxin was supplied by Lederle Laboratories.

The rabbits were observed at frequent intervals following the injection of organisms and antitoxin. The time of death was recorded. A few of the animals were sacrificed when they were moribund. A complete autopsy was performed on some of the animals. The tissues were fixed in a 4 per cent formaldehyde solution and the sections were stained with hematoxylin and eosin.

EXPERIMENTAL

Experiment 1.—The effect on rabbits of staphylococcus antitoxin given before an injection of staphylococci.

Rabbits were given a single injection of staphylococcus antitoxin four hours before 4 c.c. of an infusion broth culture of staphylococci. The results of this experiment are given in Table I. From this table it can be seen that the rabbits receiving antitoxin frequently lived a few hours longer than those in the control group.

TABLE I

This table gives the results of an experiment in which one injection of staphylococcus antitoxin (1750 units) was given four hours before the injection of 4 c.c. of a twenty-four-hour infusion broth culture of staphylococci. Both injections were intravenous.

EXPERIMENTAL GROUP			CONTROL GROUP		
NO.	WT. GM.	DEATH HR.	NO.	WT. GM.	DEATH HR.
111	2100	20	112	2400	20
113	2350	92	114	2400	56
115	1900	27½	116	2100	20
117	1700	56	118	2050	20
119	1700	127	120	1600	20

Petechiae and small abscesses were the pathologic findings in the viscera of both groups of rabbits.

Experiment 2.—The effect on rabbits of several injections of staphylococcus antitoxin given after the injection of staphylococci.

Rabbits were given staphylococcus antitoxin at the following intervals, 4½, 21, 26½, and 45 hours after 1 c.c. of a broth culture of staphylococci. The results of this experiment are given in Table II. The period of survival of the rabbits receiving the antitoxin as shown in Table II is longer than that in Experiment 1.

TABLE II

This table gives the results of an experiment in which 4 injections of staphylococcus antitoxin (1750 units each) were given 4½, 21, 26½, and 45 hours after the injection of 1 c.c. of a twenty-four-hour infusion broth culture of staphylococci. Injections were intravenous.

EXPERIMENTAL GROUP			CONTROL GROUP		
NO.	WT. GM.	RESULTS	NO.	WT. GM.	RESULTS
145	3200	Normal after 44 days	149	2300	Dead after 21 hours
146	2300	Moribund and killed after 4 days	150	3150	Dead after 45 hours
147	2200	Dead after 21 hours	151	2500	Dead after 25 hours
148	2100	Dead after 7 days			

Petechiae were the only lesions found in the control group. Rabbits 146 and 148 had abscesses in the viscera. The former rabbit also had purulent arthritis involving both elbows at the time of the autopsy.

Experiment 3.—The effect on rabbits of a single injection of staphylococcus antitoxin given before an injection of staphylococci and several injections of antitoxin given afterward.

A single injection of antitoxin was given to a group of rabbits three and one-fourth hours before 1 c.c. of a broth culture of staphylococci and at intervals of 4¼, 21¼, 24¼, and 44¼ hours after the inoculation of the organisms. The results are presented in Table III. The period of survival of the rabbits as shown here is considerably longer when the antitoxin is given before and after the injection of the bacteria.

TABLE III

This table gives the results of an experiment in which one injection of staphylococcus antitoxin (1750 units) was given three and one-fourth hours before 1 c.c. of a twenty-four-hour infusion broth culture of staphylococci and four more injections of staphylococcus antitoxin (1750 units each) were given at intervals of 4¼, 21¼, 24¼, and 44¼ hours after the injection of the organisms. Injections were intravenous.

EXPERIMENTAL GROUP			CONTROL GROUP		
NO.	WT. GM.	RESULTS	NO.	WT. GM.	RESULTS
135	2150	Dead after 39 days	138	1850	Dead after 21 hr.
136	2550	Moribund—killed after 17 days	139	1750	Dead after 31 hr.
137*	1825	Dead after 10 days	X	—	Dead after 17 hr.

*This rabbit did not receive any antitoxin before the injection of staphylococci.

The rabbits receiving the antitoxin and the broth cultures of staphylococci developed purulent arthritis between the eighth and the eleventh day of the experiment. At autopsy each of these rabbits had a few abscesses in the viscera and purulent arthritis involving one or more of the joints.

Experiment 4.—The effect on the rabbit of several injections of staphylococcus antitoxin given before and after two inoculations of staphylococci.

A single injection of antitoxin was given to a group of rabbits four and one-fourth hours before they were inoculated with a culture of staphylococci and two more injections of antitoxin were given to these animals four and twenty hours later. A second injection of organisms was made twenty-four hours after the first. Antitoxin was given fifteen minutes and nineteen hours after the second inoculation of staphylococci. The results of this experiment are shown in Table IV. The period of survival is definitely longer in the group of rabbits receiving the staphylococci and antitoxin than it is in the group given only organisms.

Petechiae and abscesses in the viscera were the characteristic lesions found in the control group of rabbits. Two of these animals at autopsy had an acute purulent arthritis involving the right knee. All the rabbits except one that received antitoxin and staphylococci had abscesses in the kidneys. Purulent arthritis was present in one or more joints of all the animals given antitoxin and staphylococci.

The supernatant from the centrifuged infusion broth culture of staphylococci showed only a 2+ hemolysis when 0.5 c.c. was added to 2 c.c. of a 1 per cent concentration of rabbit's red blood cells suspended in physiologic saline. No skin necrosis was produced by 0.1 and 0.2 c.c. of this supernatant when a rabbit, susceptible to staphylococcus toxin, was inoculated intracutaneously.

TABLE IV

This table gives the results of an experiment in which one injection of staphylococcus antitoxin (1750 units) was given three and one-fourth hours before 1 c.c. of a twenty-four-hour infusion broth culture of staphylococci and two more injections of antitoxin, four and twenty hours later. 0.5 c.c. of a twenty-four-hour infusion broth culture was given twenty-four hours after the first inoculation of organisms. Staphylococcus antitoxin was injected fifteen minutes and nineteen hours after the second injection of organisms. Injections were intravenous.

EXPERIMENTAL			CONTROL		
NO.	WT. GM.	RESULTS	NO.	WT. GM.	RESULTS
178	2800	Moribund—killed after 10 days	182	2750	Moribund—killed after 48 hr.
179	2200	Dead after 9 days	183	2900	Moribund—killed after 48 hr.
180	2200	Killed after 13 days	184*	2500	Dead after 17 hr.
181	2600	Killed after 14 days	185	3150	Dead after 48 hr.

*This rabbit received only one injection of staphylococci.

DISCUSSION

The results obtained in these experiments indicate that rabbits given infusion broth cultures of virulent staphylococci and staphylococcus antitoxin intravenously live for a longer period than rabbits given the same quantity of organisms and no antitoxin. The length of time between the inoculation of staphylococci and antitoxin and the amount of antitoxin also influence the length of the survival period.

The absence of abscesses in the viscera of those animals that died within twenty-four hours after the inoculation of staphylococci and antitoxin or only staphylococci, suggest that death is the result of the action of a toxin produced in vivo. The amount of hemotoxin and skin necrotizing toxin in the bacterial inoculum was insufficient to cause death. Certain strains of staphylococci apparently produce toxin in vivo during the first twenty-four hours after the intravenous injection of an infusion broth culture eighteen to twenty-four hours old. Staphylococcus antitoxin apparently inhibits the action of this toxin; however, it does not prevent the localization of staphylococci and the subsequent development of abscesses in the viscera and purulent arthritis.

The frequency of purulent arthritis is greater in those rabbits given staphylococci and antitoxin than it is in those animals receiving only staphylococci.

Staphylococcus toxin and antitoxin react the same as other toxins and antitoxins. Ramon and others[7] are of the opinion that staphylococcus antitoxin is also indirectly bactericidal. These investigators state that the antitoxin neutralizes the leucociden in staphlyococcus toxin and this prevents a destruction of the phagocytic polymorphonuclear leucocytes. The tissues are protected against the necrotizing action of the staphylococcus poison and also there is a less favorable medium for the growth and multiplication of organisms.

The results obtained in this experiment and the observations of others[4, 5, 8, 9] show conclusively that staphylococcus antitoxin prolongs the period of survival following the intravenous injection of broth cultures of virulent staphylococci.

SUMMARY

Staphylococcus antitoxin prolongs the period of survival in rabbits inoculated intravenously with broth cultures of a virulent strain of *Staphylococcus aureus*. Antitoxin does not prevent the localization of staphylococci in the viscera and the joints.

Rabbits that died within twenty-four hours after receiving a broth culture of virulent staphylococci apparently succumb to the effect of a toxin produced in vivo.

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LABORATORY METHODS

THE PRESERVATION OF SPUTUM FOR PNEUMOCOCCUS-TYPE DIFFERENTIATION*

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IN TEACHING or demonstrating the Neufeld technic, the use of sputum from patients with pneumonia has been found essential. A specimen prepared by the artificial admixture of a culture of pneumococci and sputum is never realistic, nor does the microscopic appearance of such preparations bear a very close resemblance to an actual specimen from a pneumonia patient. Since fresh sputum is not always available, however, and pneumococci disintegrate after a short period and are overgrown by contaminating bacteria and molds, the desirability of using a preservative becomes evident.

Etinger-Tulezynska[1] noted that heat-killed pneumococci retained the capacity of developing the *Quellungsreaktion*, and Bullowa[2] states that "The Neufeld reaction has been obtained . . . in some (sputums) inadvertently preserved in solution of formaldehyde, tricresol or phenol." Since chemical treatment seemed to afford a simple and convenient means of preservation, an investigation was undertaken to determine the effect of adding phenol, "Merthiolate," glycerol, and formalin to specimens from pneumonia patients which had been mailed to the laboratory. Nineteen such specimens were included in the study; ten contained Type I pneumococci, two Type II, three Type III, three Type VIII, and one Type XIV.

Pneumococci in portions of sputum to which had been added varying amounts of 0.5 per cent phenol disintegrated more rapidly than those in the control portions without preservative. In "Merthiolate," 1:1000 and 1:5000, the cell bodies of the pneumococci began to lose their normal structure after a few days. Accordingly, work with these two chemicals was discontinued. It was hoped that 30 per cent glycerol might prove effective, since this solution does not kill the microorganisms as do the other preservatives. However, the viability of pneumococci and the capacity for developing the *Quellungsreaktion* persisted no longer in the glycerolated portions than in the unpreserved controls nor were the treated specimens suitable for mouse inoculation because of the toxic effect of the preservative. A 1 per cent solution of formalin (40 per cent formaldehyde) proved very effective in preserving both the cellular structure and the "*Quellung*" capacity of the pneumococci in all of the specimens

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examined, except those containing pneumococci Type III. In this type, the duration of the property of "capsular swelling," which normally cannot be demonstrated after a few days, was not prolonged by the addition of preservative. Generally, when pneumococci in sputum disintegrate, the cell bodies disappear first, leaving what appears to be an empty capsule which is still capable of developing the *Quellungsreaktion*. Welch[3] described such forms in the fluid of empyema following pneumonia. On the other hand, the Type III pneumococci in the specimens studied behaved as do pneumococci in broth cultures in that their capsules disappeared first, as was indicated by loss of the property of yielding a typical reaction in homologous serum. To determine whether the fixing of pneumococci would result in the development of nonspecific capsular swelling in the presence of heterologous serums, control tests were performed in a large number of instances, but no nonspecific reactions were obtained.

Contrary to the opinion expressed in the literature,[4] and which is quite generally accepted, pneumococci, with the exception of Type III, can usually be demonstrated in sputum after it has been kept for several days at room temperature or in the ice box, and sometimes even after forty-eight hours in the incubator, provided the specimen consists of exudate that has been coughed up and has not been contaminated. The "*Quellung*" capacity appears to remain for a considerable time after the microorganisms have lost their viability. These facts, to be sure, do not modify the cogent necessity of prompt examination of pneumonic sputum submitted for diagnosis. There is no need for the preservation of such specimens; indeed, killing the pneumococci renders them unfit for confirmatory cultural examination and animal inoculation and should therefore be avoided.

The specimens of sputum in formalin, with the exception of those containing Type III pneumococci, were used for more than four months for teaching and exhibition purposes. At the end of that time, the majority of the pneumococci in each specimen were still normal in appearance and developed the characteristic *Quellungsreaktion* in the presence of hemologous typing serum. After varying periods, the unpreserved specimens and those to which glycerol had been added became so overgrown with molds that they were discarded.

The method of preservation consists of adding to a portion of sputum a 1 per cent solution of formalin in an amount at least twice that of the specimen and storing the mixture at room temperature. Preliminary centrifugation was not found necessary in typing the specimen. A fleck of sputum can easily be fished with a platinum loop. Even after the sputum has become liquefied through dissolution of the fibrin, the microorganisms can be found in the sediment at the bottom of the jar.

SUMMARY

The preservation of sputum from pneumonia patients by the addition of 1 per cent formalin has proved advantageous in preparing specimens for prolonged use in teaching and for exhibition purposes. Satisfactory preservation has been accomplished with specimens containing pneumococci Types I, II, VIII.

and XIV, but not with those containing Type III. Phenol, "Merthiolate," 1:1000 and 1:5000, and 30 per cent glycerol were found to be unsatisfactory for this purpose.

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THE DETERMINATION OF PLATELET VOLUME*

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THE volumetric measurement of the blood platelets is beginning to occupy an important place in hematology. The determination of the volume of packed platelets has been proposed by some investigators, Fowler,[1] Evans and Fowler,[2] and Rivasi[3] among others, as a substitute for platelet enumeration. According to Bayne-Jones[4] and Tait and Green[5] unruptured thrombocytes constitute prothrombin. The determination of the platelet volume, therefore, offers a method for obtaining the total quantity of available prothrombin in a given blood. This procedure may be employed also for studying some of the functional properties of the platelets since it has been pointed out by Zeller,[6] Barta,[7] Jürgens and Naumann,[8] and Jürgens[9] that there is a definite relationship between the size of the thrombocytes and the functions they exhibit.

METHOD

Principle of Method.—It is well known that the platelets immediately upon their extravascular escape begin to undergo disintegration, the rate of this disintegration depending on several factors such as the nature of the blood diluent employed, the degree of dilution, the temperature of the platelet suspension, and the length of time the platelets are allowed to remain suspended in the diluent. Obviously, by employing a satisfactory platelet preservative and by rapidly isolating the platelets after the blood has been diluted, the loss of any significant quantity of thrombocytes can be avoided. In this method the separation of the platelets from the remaining formed blood elements is quickly accomplished by centrifuging the diluted blood. This procedure for obtaining a pure platelet suspension was introduced by Mosen[10] and has recently been employed by Fonio[11] for fractional blood transfusion and by Fonio[12] and Leslie and Sanford[13] for studying the functional properties of the platelets.

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When blood is diluted with an anticoagulant as described below and then centrifuged for one and one-half minutes at 2000 r.p.m. the blood suspension becomes separated into three layers as shown in Fig. 1.

Layer P (platelet layer).—This layer has a whitish, ground-glass appearance and with the dilutions employed here occupies a volume of about 7.5 c.c. It consists almost entirely platelets in lower portion of this layer (level P_2) is 15 to 25 per cent greater than in its upper of platelets; leucocytes and erythrocytes are but rarely seen. The concentration of the portion (level P_1). In determining the platelet volume it is important to remove most of this layer into the thrombocytocrit pipette in order to reduce the loss of platelets to a minimum.

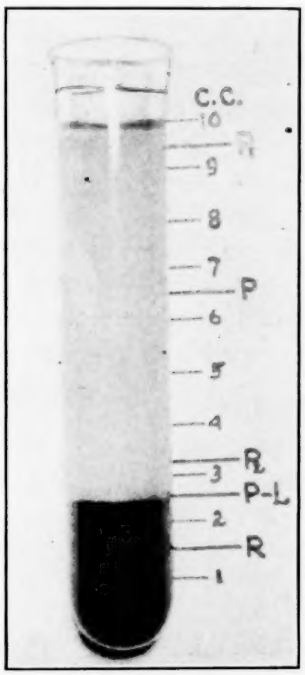

Fig. 1.—Appearance of layers formed by the cellular blood elements in a centrifuge tube after centrifugalization for one and one-half minutes. *P*, platelet layer; *P-L*, platelet-leucocyte layer; *R*, red cell layer.

Layer P-L (platelet-leucocyte layer).—The appearance and composition of this intermediate layer varies considerably, depending on the suspension stability of the erythrocytes, leucocytes, and platelets and on the rate and duration of centrifugation. Usually this layer consists of a very thin, grayish white, semi-transparent membrane. Instead of this distinct membrane there may be formed a hazy, ill-defined, reddish gray layer 1 or even 2 mm. thick. When well-defined this intermediate layer consists mostly of leucocytes and platelets. Occasionally after centrifuging there is formed instead a well-marked, nontransparent, membrane about 0.25 mm. thick. Such an intermediate layer indicates considerable concentration of large quantities of platelets at this level. The time of centrifuging is then best reduced to one and one-quarter minutes.

Layer R (Red Cell Layer).—This layer consists of numerous red blood cells, occasional white blood cells (4,000 to 8,000 per c.mm.) and platelets (about 3,000 per c.mm.). It occupies a volume of about 2.5 c.c. The quantity of platelets found among the red cells varies inversely with the speed of centrifugalization as shown in Table I.

TECHNIC

Apparatus and Solutions Required.—1. Electric centrifuge with an arm length of about 20 cm. and capable of delivering 4000 r.p.m. In this study an International electric centrifuge, size 1, Type C, was employed for isolating the platelets, and a special centrifuge for the final platelet volume determination.

TABLE I

EFFECT OF CENTRIFUGALIZATION ON CONCENTRATION OF PLATELETS IN RED CELL LAYER

CENTRIFUGE SPEED, R.P.M.	PLATELETS PER C.MM. IN RED CELL LAYER
300	52,000
1,000	36,000
2,000	3,000

2. Centrifuge tubes of 15 mm. outside diameter, 75 to 80 mm. long and approximately 11 c.c. capacity. These can be made from standard Pyrex test tubes (15 by 125 mm.) by cutting off a segment 45 to 50 mm. long near the open end.

3. Thrombocytocrit pipettes (Van Allen) with a capacity of at least 7.5 c.c.

4. Standard 10 c.c. syringe and needles for venipuncture.

5. Tuberculin or standard 1.5 c.c. syringe.

6. Five per cent solution of olive oil in ether.

7. Platelet preservative. The blood diluent employed here has the following composition:

	GM. OR C.C.
Sodium metaphosphate (Howe and French)	1.0
Sodium chloride	0.5
Dextrose	0.1
Distilled water	100.0

All glassware must be thoroughly cleaned with a mixture of sulphuric acid and potassium bichromate, then washed with distilled water and finally dried with alcohol and ether.

The mixture of olive oil and ether is drawn through the venipuncture needle and the ether allowed to evaporate. The needle is attached to a 10 c.c. syringe which is then filled with exactly 7 c.c. of diluent. By venipuncture exactly 3 c.c. of blood are drawn into the same syringe, care being taken that no air bubbles are caught in the mixture. The use of the tourniquet should be avoided. The blood and diluent are intimately mixed by inverting the syringe several times. Two or three small drops are then allowed to escape into a special paraffin cup containing a few drops of the same preserving fluid, and a total and differential platelet counts obtained by the methods described by me elsewhere.[14, 15] The fluid remaining in the syringe is then expelled into a centrifuge tube. The latter is centrifuged for one and one-half minutes at 2000 r.p.m. As much as possible of the supernatant platelet suspension (layer *P*, Fig. 1) is then drawn by mouth suction into a thrombocytocrit pipette, being careful not to include any red cells at the same time, and the sealing clip applied to the pipette. A little of the red cell sediment is then removed from the centrifuge tube by

means of a small syringe and needle and a small drop or two injected into the thrombocytocrit pipette through its upper open end. The pipette is then centrifuged for one and one-half hours at a rate of 4000 r.p.m. At the end of this period of time the formed blood elements become separated into two sharply defined columns, an upper one consisting entirely of platelets, and a lower one consisting of red cells. The volume of the platelets contained in the original 3 c.c. of blood is then read directly on the scale of the thrombocytocrit pipette, and from the reading obtained the volume of packed platelets in 100 c.c. of blood computed. Example: a thrombocytocrit reading of 0.01 c.c. indicates the quantity of platelet substance in 3 c.c. of blood; 100 c.c. of the same blood contains, therefore, 0.33 c.c. of packed platelets, yielding a platelet volume of 0.33 volumes per cent.

COMMENTS ON THE METHOD

It is essential to avoid the inclusion of any red cells in the platelet suspension while the latter is being drawn into the thrombocytocrit pipette. If red cells are included in the platelet suspension then the platelet column in the thrombocytocrit pipette at the end of the centrifugation period is nearly always pink instead of ivory white and always yields higher readings. On microscopic examination such a column reveals, in addition to the platelets, frequent erythrocytes and leucocytes. If the technic described here is followed closely the final platelet column in the thrombocytocrit tube is always found to be ivory white and on microscopic examination reveals numerous well-preserved platelets, only rare white blood cells and no red cells. However, in order to avoid the inclusion of any red cells while removing the platelet suspension with the thrombocytocrit pipette a small quantity (usually 0.2 to 0.3 c.c.) must be left in the centrifuge tube. When the intermediate layer is well defined, then usually more of the platelet suspension can be removed without disturbing the red cell layer than when this zone is not sharply demarcated. With normal blood diluted as described above there is usually formed in the centrifuge tube at the end of one and one-half minutes about 7.5 c.c. of platelet suspension and about 2.5 c.c. of packed red cells. In the average determination 0.3 c.c. of the platelet suspension is left in the centrifuge tube and thus excluded from the final platelet volume determination. The loss of this quantity of fluid would incur an error of 4 per cent on the assumption that the platelets are evenly suspended in the plasma column (layer P, Fig. 1). Actually, however, the lower levels (level P_2, Fig. 1) of this layer contain 15 to 25 per cent more platelets per unit volume than the upper levels (level P_1, Fig. 1). Assuming the maximum difference in the concentration of the platelets between the upper and lower levels to be 25 per cent, there is then produced an additional error of 1 per cent. The total maximum error, therefore, resulting from the loss of platelet plasma left in the centrifuge tube is 5 per cent.

There are about 3000 platelets per c.mm. among the red cells in layer R, Fig. 1. This quantity of platelets is too small to yield a volume that could be measured in the thrombocytocrit pipette. The error, therefore, due to the loss of platelets present in this layer is negligible.

According to Wintrobe[16] the average volume of the cells of the myeloid series is about 25 times greater than the average volume of the platelets. From the observations of Horwitz,[17] it would seem that this difference is of considerably greater magnitude. The leucocytes, therefore, might constitute a source of considerable error if enough of them are retained in the platelet suspension. The concentration of the leucocytes in the red cell layer (layer *R*, Fig. 1) is usually about the same as in venous blood. After centrifuging the diluted blood for one and one-half minutes, the volume occupied by the red cell sediment is usually about 2.5 c.c. Five-sixths of the total number of leucocytes contained in the original 3 c.c. of blood, therefore, are to be found among the packed erythrocytes, while most of the remaining white blood cells are deposited in considerable numbers in the intermediate layer (layer *P-L*, Fig. 1) where their concentration per unit volume is 2 to 3 times greater than in venous blood. The plasma layer (layer *P*, Fig. 1), then, contains very few leucocytes and the error in the final platelet volume determination resulting from this source is, therefore, slight.

Table II shows the results obtained in 31 normal adults. The lowest platelet volume was 0.26 per cent, the highest 0.44, and the average for the entire group 0.33.

In order to test the accuracy of the method simultaneous platelet volume determinations were carried out on specimens of blood obtained from the right

TABLE II

VOLUME OF PACKED PLATELETS IN NORMAL ADULTS

NO.	SEX	AGE	PLATELETS PER C.MM.	VOLUME OF PACKED PLATELETS PER 100 C.C. BLOOD
1	M	21	510,000	0.30
2	F	45	403,000	0.26
3	M	60	481,000	0.32
4	M	35	428,000	0.32
5	F	40	471,000	0.26
6	M	22	509,000	0.33
7	M	32	499,000	0.34
8	F	40	485,000	0.36
9	M	22	456,000	0.28
10	M	36	406,000	0.35
11	M	22	411,000	0.38
12	M	38	406,000	0.34
13	M	36	433,000	0.28
14	M	36	466,000	0.39
15	M	35	461,000	0.32
16	M	30	451,000	0.29
17	M	60	490,000	0.33
18	M	21	377,000	0.31
19	M	50	400,000	0.28
20	M	34	483,000	0.33
21	M	31	361,000	0.28
22	M	34	460,000	0.34
23	M	30	426,000	0.29
24	M	45	480,000	0.35
25	F	19	378,000	0.37
26	M	19	470,000	0.27
27	M	55	490,000	0.35
28	F	41	510,000	0.27
29	M	37	497,000	0.43
30	M	20	481,000	0.41
31	F	43	521,000	0.44

and left arms of the same subject. As shown in Table III the maximum percentage of variation was 10.3.

Some investigators maintain that there is a parallelism between the volume of packed platelets and the total thrombocyte count. According to Jepsen,[18] for example, a count of 100,000 to 200,000 platelets per c.mm. will yield a platelet volume of 0.04 to 0.05 per cent; 200,000 to 300,000, 0.25 per cent; 300,000 to 400,000, 0.42 per cent, etc. I have failed to observe such a close parallelism between the platelet volume and the total platelet count. In fact, Jepsen himself reported four normal adults with counts of 530,000, 420,000, 310,000 and 410,000 platelets per c.mm. with corresponding platelet volumes of 0.26, 0.34, 0.50 and 0.46 per cent. There is no doubt that, in general, the volume of packed platelets depends on the total thrombocyte count, as shown in Table IV. The correlation, however, is not a close one. This is illustrated by Cases 1, 28, and 31, Table I, which yielded platelet volumes of 0.30, 0.27, and 0.44 per cent, respectively, despite the almost identical total platelet counts.

TABLE III

SIMULTANEOUS DETERMINATIONS OF THE VOLUME OF PACKED PLATELETS IN SPECIMENS OF BLOOD OBTAINED FROM THE RIGHT AND LEFT ARMS OF THE SAME SUBJECT

SEX	AGE	PLATELETS PER C.MM.	VOLUME OF PACK PLATELETS		MAXIMUM PERCENTAGE OF VARIATION	DIAGNOSIS
			ARM			
			RIGHT	LEFT		
M	30	377,000	0.31	0.32	3.2	Normal
M	60	481,000	0.32	0.29	10.3	Normal
M	36	406,000	0.35	0.34	2.9	Normal
M	30	451,000	0.29	0.29	0.0	Normal
M	35	461,000	0.32	0.34	6.2	Normal
F	25	807,000	0.48	0.44	8.3	Postinfectious thrombocytosis
F	50	645,000	0.40	0.42	5.0	Pernicious anemia under liver treatment
M	59	217,000	0.16	0.16	0.0	Hemochromatosis
F	26	753,000	0.45	0.43	4.4	Neuritis
F	60	1,180,000	0.83	0.90	8.4	Chronic myelogenous leucemia

These discrepancies between the total thrombocyte counts and the corresponding platelet volumes are most likely due to the quantitative changes in the thrombocytes occurring during the process of determination of the volume of packed platelets. The sodium metaphosphate solution employed here as blood diluent preserves the platelets well for about two hours. The average determination of the platelet volume takes less than two hours. However, some of the platelets do become disintegrated after having been subjected to a centrifugation force of 4000 r.p.m. for one and one-half hours. Indirect evidence for this disintegration of the thrombocytes is afforded by the changes in their differential formula. The method for obtaining differential platelet counts is described by me in detail elsewhere.[15] Briefly, the platelets as observed in wet preparations, can be separated into four groups according to size: Group I, consisting of platelets the diameter of which is one-quarter that of a red cell, or about 1.8 microns; Group II, consisting of platelets with a diameter one-third that of a red cell, or about 2.5 microns; Group III, consisting of platelets with a diameter one-half that of a red cell or greater, or about 3.6 microns; and Group IV, con-

sisting of irregularly-shaped platelets. With normal capillary blood obtained from the finger the usual formula is: Group I, about 19 per cent; Group II, about 63 per cent; Group III, about 17 per cent; Group IV, about 1 per cent. With venous blood the results are somewhat different. In the 31 normal adults

TABLE IV

RELATION OF VOLUME OF PACKED PLATELETS TO TOTAL THROMBOCYTE COUNT

SEX	AGE	PLATELETS PER C.MM.	VOLUME OF PACKED PLATELETS	DIAGNOSIS
		452,000	0.33	Average, 31 normals
M	36	406,000	0.35	Normal
		202,000	0.17	After daily injection of gm. 1 amidopyrine for 3 days
F	17	19,000	0.01	Thrombocytopenic purpura
M	59	217,000	0.16	Hemochromatosis
M	60	730,000	0.50	Postinfectious thrombocytosis
F	60	1,180,000	0.86	Chronic myelogenous leucemia

in Table I the averages were: Group I, 30 per cent; Group II, 60 per cent; Group III, 7 per cent; Group IV, 3 per cent. I have pointed out elsewhere[19] that during the usual course of the disintegration of the platelets the larger thrombocytes, those belonging to Groups III and IV, break up into two or more fragments which then become members of either Group I or II; the platelets of Group II in turn disintegrate to become units of Group I. A change in the differential formula, therefore, characterized by a shift to the left due to an increase in the number of the smaller platelets indicates that some of the larger platelets have at least partly disintegrated. To illustrate this point venous blood was drawn from two normal adults and differential platelet counts carried out (1) immediately after drawing the blood, (2) after having centrifuged the diluted blood for one and one-half minutes in order to isolate the platelets, and (3) after having centrifuged the platelet suspension for one and one-half hours at 4000 r.p.m. for the platelet volume determination. The results are shown in Table V. The determination of the volume of packed platelets, therefore, involves an unavoidable disintegration and consequent loss of some of the thrombocytes.

TABLE V

CHANGES IN THE DIFFERENTIAL PLATELET FORMULA DURING THE DETERMINATION OF THE VOLUME OF PACKED PLATELETS

	DIFFERENTIAL PLATELET FORMULA (PER CENT)				TIME
	GROUP I	GROUP II	GROUP III	GROUP IV	
Subject A	32	59	2	7	Immediately after drawing blood
	25	52	5	18	After centrifuging the diluted blood for one and one-half minutes
	40	27	0	33	After centrifuging platelet suspension for one and one-half hours
Subject B	32	60	8	0	Immediately after drawing blood
	32	57	2	9	After centrifuging the diluted blood for one and one-half minutes
	54	33	1	12	After centrifuging platelet suspension for one and one-half hours

However, it is the variation in the rate of this disintegration of the platelets during the course of the determination of the volume of packed thrombocytes which produces the discrepancies observed between the platelet volumes and the total thrombocyte counts. In Cases 1, 28, and 31 (Table I), previously cited, the rates of disintegration of the platelets determined by the method described by me elsewhere[19] were 12.4, 25, and 10 per cent, respectively, with corresponding platelet volumes of 0.30, 0.27, and 0.44 per cent. A disintegration index of 10 per cent (indicating that 10 per cent of the platelets have become completely disintegrated during the observation period) shows unusual stability of the platelets in capillary or venous blood, and accounts for the rather high platelet volume in Case 31. In other words, there seems to be an inverse relationship between the volume of packed platelets and their fragility, thrombocytes of greater stability yielding higher platelet volumes.

The volume of packed platelets is at times influenced by the differential platelet formula. From Table VI it is obvious, however, that the differential formula must be grossly abnormal in order to influence the platelet volume to a significant degree and that a shift to the left due to an increase in the number of the smaller types of platelets is much less important than a shift to the right resulting from an increase in the number of the larger thrombocytes. This is illustrated by Case 4, Table VI. Despite the thrombopenia present, the volume of packed platelets was about normal. The differential formula in this case revealed numerous large and giant platelets, many the size of a red cell or larger.

TABLE VI

EFFECT OF VARIATIONS IN THE DIFFERENTIAL PLATELET FORMULA ON THE VOLUME OF PACKED PLATELETS

CASE	SEX	AGE	PLATELETS PER C.MM.	DIFFERENTIAL PLATELET FORMULA (PER CENT)				PLATELET VOLUME	DIAGNOSIS
				GROUP I	GROUP II	GROUP III	GROUP IV		
			452,000	30	60	7	3	0.33	Average, 31 normals
1	M	38	406,000	19	68	11	2	0.34	Normal
2	M	22	411,000	32	61	4	3	0.38	Normal
3	M	59	217,000	12	44	43	1	0.16	Hemochromatosis
4	M	30	98,000	1	12	83	4	0.26	Chronic thrombocytopenic purpura

The mean or average volume of a single platelet may be determined by employing Wintrobe's[20] method for obtaining the mean corpuscular volume of red cells. In the 31 normal adults studied the mean platelet volume was found to be 7.3 cubic microns. Horwitz[17, 21] found the average platelet volume to be 5 cubic microns; Nygaard,[22] 12 cubic microns; Wintrobe,[16] 30 cubic microns. Wintrobe's value is too high. Macrothrombocytes of that size I have observed only in a case of chronic thrombocytopenic purpura (Case 4, Table VI) in which the average platelet volume was 26.5 cubic microns.

VAN ALLEN'S METHOD

At the present time the method usually employed for determining the volume of packed platelets is that of Van Allen.[23] The principle on which the method is based is described by the author as follows: "If a sample of blood, freshly

drawn and mixed with an anticoagulant, be allowed to stand, the corpuscles will sink gradually and leave a supernatant fluid of whitish, ground-glass appearance in which the platelets remain uniformly suspended for hours. As is well-known, the platelet content of this fluid is the same per unit volume as was that of the whole specimen before sedimentation, and this fact has been made use of in the platelet counting method of Thomsen."

Van Allen, in presenting the principles of his method, assumes (1) that the platelet plasma contains per unit volume the same quantity of platelets as did the whole specimen of blood before sedimentation and (2) that the platelets remain uniformly suspended for hours. It was Bürker[24, 25] who was first to observe that when blood is allowed to stand, the platelets "rise" to the upper levels while the heavier formed blood elements, the leucocytes and erythrocytes, sink to the lower levels. Thomsen's[26, 27] method for enumerating thrombocytes is based on this observation. Thomsen, however, never maintained that the

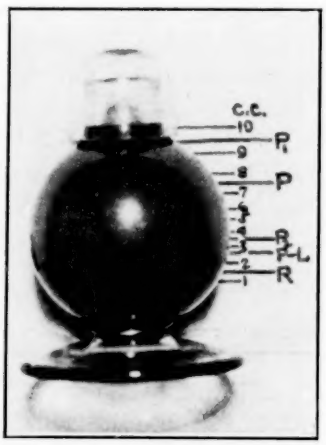

Fig. 2.—Appearance of layers formed by the cellular blood elements in a Van Allen sedimentation chamber at the end of three and one-half hours. *P*, platelet layer; *P-L*, platelet-leucocyte layer; *R*, red cell layer.

concentration of the platelets in the plasma layer after the diluted blood has been allowed to stand for several hours is the same as of the whole specimen of blood before sedimentation. He merely stated that the platelets remain evenly suspended in the plasma layer for several hours. If a Van Allen sedimentation chamber is filled with blood diluted as specified by the author and allowed to stand undisturbed for three and one-half hours, the specimen becomes separated into layers as shown in Fig. 2. These layers are quite similar to those illustrated by Fig. 1. The red cell layer R varies considerably in quantity, depending on the rate of sedimentation of the erythrocytes. With a sedimentation chamber of 10 c.c. capacity normal blood yields a red cell column whose volume is 2.5 to 6 c.c. When the diluted blood is placed in the sedimentation chamber, the platelets quickly "rise" to the upper levels, as was pointed out by Bürker, while the leucocytes and erythrocytes sink to the lower levels. If the sinking velocity of the erythrocytes is rather slow, a good many platelets may be found among them at the end of three and one-half hours, the concentration of the

platelets per unit volume, however, never exceeding one-half the concentration of the platelets found in the overlying plasma layer. With normal blood, however, at the end of the sedimentation period the quantity of platelets found among the red cells is usually so scanty (less than 50,000 per c.mm.) that it can be disregarded for practical purposes. This is in agreement with the observations of Horwitz.[17] Van Allen's results are, therefore, inaccurate since they fail to include the correction for the volume of the packed red cells.

Thomsen and a number of other investigators employing his procedure maintain that the platelets remain evenly suspended in the plasma for several hours, at least six according to Thomsen himself. If blood, diluted or undiluted, is placed in a sedimentation chamber, the platelets quickly rise to the upper levels of the fluid and form a suspension which is probably fairly uniform in the beginning. After a variable length of time, however, some of the platelets begin to sink to the lower levels of the plasma layer, the rate of sedimentation of the platelets depending on several factors such as their size, the magnitude of their electric charge and the composition of the medium in which they are suspended.[8, 9, 28, 29] I have observed that when blood is diluted with isotonic sodium oxalate solution as described by Van Allen and placed in a Van Allen sedimentation chamber, at the end of three and one-half to four hours the concentration of the platelets in the plasma layer at level P_2 (Fig. 2) may be 56 per cent greater than at level P_1. It would seem, therefore, that the platelet suspension is rather unstable. The observations of Jürgens,[28] Baunack,[30] and Windfeld[31] corroborate this view.

In determining the volume of packed platelets it is essential to take into consideration the variation in the concentration of the platelets in the various levels of the plasma layer. Van Allen allows the oxalated blood to stand for three and one-half hours, then places 5 c.c. of the supernatant platelet suspension in a thrombocytocrit pipette. Obviously, if the upper portion of the plasma column is removed for the determination of the platelet volume, the results will be too low; if the lower portion of the plasma column is used, the results will be too high. The error introduced by this factor can be obviated, however, by placing all of the available platelet suspension in the thrombocytocrit pipette. Even then Van Allen's method in my hands yielded unsatisfactory results. I repeated his method by using a 10 c.c. sedimentation chamber and removing as much as possible of the platelet suspension into the thrombocytocrit pipette at the end of three and one-half hours. The volume of the packed platelets was computed

TABLE VII

COMPARISON OF THE VOLUMES OF PACKED PLATELETS DETERMINED BY THE AUTHOR'S AND VAN ALLEN'S METHODS

CASE	VOLUME OF PACKED PLATELETS	
	AUTHOR'S METHOD	VAN ALLEN'S METHOD
1	0.30	0.20
2	0.26	0.12
3	0.42	0.12
4	0.32	0.12
5	0.50	0.25

TABLE VIII

COMPARATIVE OBSERVATIONS OF THE TOTAL AND DIFFERENTIAL PLATELET COUNTS AND THE RATE OF DISINTEGRATION OF PLATELETS IN SODIUM METAPHOSPHATE AND ISOTONIC SODIUM OXALATE SOLUTIONS

SOLUTION	PLATELETS PER C.MM.				DIFFERENTIAL PLATELET FORMULA (PER CENT). CAPILLARY BLOOD								DIFFERENTIAL PLATELET FORMULA (PER CENT) VENOUS BLOOD							
	CAPILLARY BLOOD		VENOUS BLOOD		GROUP I		GROUP II		GROUP III		GROUP IV		GROUP I		GROUP II		GROUP III		GROUP IV	
	0 HR.	3½ HR.	0 HR.	3½ HR.	0 HR.	3½ HR.	0 HR.	3½ HR.	0 HR.	3½ HR.	0 HR.	3½ HR.	0 HR.	3½ HR.	0 HR.	3½ HR.	0 HR.	3½ HR.	0 HR.	3½ HR.
Subject A:																				
Sodium metaphosphate	424,000	345,000	434,000	316,000	22	27	62	70	7	2	9	6	32	46	59	46	2	3	7	5
Isotonic sodium oxalate	240,000	76,000	132,000	81,000	29	61	64	39	7	0	0	0	48	92	45	8	6	0	1	0
Subject B:																				
Sodium metaphosphate	438,000	273,000	392,000	245,000	27	39	65	55	8	6	0	0	32	40	60	48	8	11	0	1
Isotonic sodium oxalate	286,000	166,000	377,000	80,000	36	50	53	48	3	0	8	2	30	67	63	20	0	0	7	13

as illustrated by the following example: reading on the scale of the thrombocytocrit pipette 0.0025 c.c., indicating 0.0025 c.c. of platelet substance in 2 c.c. of blood; the platelet volume is therefore 0.12 per cent. The results were surprisingly low as shown in Table VII.

Van Allen allows the blood oxalate solution to stand for three and one-half hours. During this period of time several significant changes take place in the total and differential platelet counts, as shown in Table VIII. All the determinations recorded in this table were carried out simultaneously by using the bloods from two normal adults. The table demonstrates three features. First, the total platelet counts were lower when sodium oxalate was employed as blood diluent. The oxalate solution, obviously, is not as good a platelet preservative as the sodium metaphosphate solution. Second, the differential platelet counts in the sodium oxalate solution revealed higher percentages of the smaller types of platelets. This is most likely due to the fact that the larger platelets break up readily in this solution. Third, after the blood diluted with the sodium oxalate solution has been allowed to stand for three and one-half hours, a variable, but usually quite considerable, quantity of thrombocytes becomes entirely disintegrated and is therefore not included in the determination of the platelet volume.

Van Allen's method, in my opinion, is unsatisfactory. The sodium oxalate solution employed here as blood diluent is a poor platelet preservative. This is especially evident from the examination of the platelet column in the thrombocytocrit pipette at the end of the centrifugalization period: the platelets seem to have lost their normal refractile properties and appear to lie imbedded in granular débris representing, no doubt, partially disintegrated platelets. The method involves a number of compensatory errors, some tending to elevate the final result, others to lower it.

SUMMARY AND CONCLUSIONS

A method is described for the determination of the volume of packed platelets.

In a series of 31 normal adults the lowest platelet volume was 0.26, the highest 0.44, and the average 0.33 volumes per cent.

The mean platelet volume (average volume of a single platelet) in the 31 normal adults studied was found to be 7.3 cubic microns.

The volume of packed platelets is dependent chiefly on the total thrombocyte count. The occasional lack of absolute parallelism between the volume and the total count of the platelets may be attributed mainly to the variations in the fragility and size of the thrombocytes.

The determination of the volume of packed thrombocytes is a useful hematologic procedure. It should not, however, be employed as a substitute for platelet enumeration.

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THE DETERMINATION OF TOTAL PROTEIN, SUGAR AND CHLORIDES IN CEREBROSPINAL FLUID*

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FOR the past three years we have used a procedure for the determination of total protein, sugar and chlorides in spinal fluid, which is simple and accurate. Since these are most usually examined for, it was thought worth while to report the procedure in detail. All three of the determinations may be made on as little as 1 c.c. of spinal fluid.

PROCEDURE

Pipette 1 c.c. of spinal fluid into a 15 c.c. graduated centrifuge tube, add 8 c.c. of distilled water, 0.5 c.c. of the tungstomolybdic precipitating reagent of Benedict,[1] and 0.5 c.c. of ⅔ N sulphuric acid. Mix by inversion, and allow to stand for fifteen minutes for precipitation of the proteins. Centrifuge the tube at high speed for five minutes. All of the protein will be precipitated in the tip of the tube, and a water clear supernatant fluid will be obtained. The clear supernatant fluid is decanted into a test tube and is used for the determination of sugar and chlorides.

Determination of Sugar.—Two cubic centimeters of the supernatant fluid (equivalent to 0.2 c.c. spinal fluid), is transferred to a Folin sugar tube, and 2 c.c. of copper sulphate solution added, exactly as directed by the Benedict[2] procedure for blood sugar. The final dilution should be 12.5 c.c. instead of 25 c.c. The result obtained divided by 2 gives the spinal fluid sugar concentration.

Chlorides.—Five cubic centimeters of the supernatant fluid (equivalent to 0.5 c.c. of spinal fluid), is used for the chloride estimation. The Whitehorn[3] method as applied to the determination of blood chlorides is used.

Total Protein.—To the precipitate remaining in the centrifuge tube (i.e., the protein precipitated from 1 c.c. of spinal fluid) add 2 drops of 10 per cent sodium hydroxide solution, agitate until dissolved; with the aid of two 1 c.c. portions of water transfer the dissolved protein to a Pyrex test tube 7 by 1 inch, with a constriction two inches from the bottom, the constricted portion being ¾ inch in diameter.† Place a glass bead in the tube, and add 1 c.c. of nitrogen free concentrated sulphuric acid, and heat the tube over a microburner until the mixture starts to turn black, then add 2 or 3 drops of 30 per cent hydrogen peroxide, continue heating until the mixture becomes clear, adding more hydrogen peroxide if necessary. When cool, add 2 or 3 c.c. of distilled water, 5 c.c. of a 40 per cent sodium hydroxide solution, and 4 drops of capryllic alcohol, place in the rear tube of the aeration apparatus (such as is used for

*From the Achelis Laboratory, Lenox Hill Hospital.
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†These tubes may be obtained from Eimer & Amend, N. Y.

urea nitrogen determinations in blood), and aerate into a tube containing 10 c.c. of distilled water and three drops of 10 per cent hydrochloric acid for one-half hour. When aeration is complete transfer the contents of the receiving tube to a graduated cylinder, add 10 c.c. of diluted Nessler's solution and dilute to 25 c.c. with water,* and compare in the colorimeter with a suitable standard (usually 5 c.c. equivalent to 0.5 mg. nitrogen). To convert the nitrogen into protein multiply by the usual factor 6.25.

SUMMARY

A simple procedure is outlined for the determination of the sugar, chlorides and total protein in spinal fluid. All determinations can be made upon 1 c.c. of fluid.

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A MODIFICATION OF THE TECHNIC OF THE NEUFELD REACTION IN THE TYPING OF PNEUMOCOCCI†

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SINCE the advent of the Neufeld reaction for the typing of the pneumococci we have had a simple laboratory procedure useful in any laboratory large or small with a great degree of accuracy. However, there are times when, in examining the specimen, a certain amount of débris, other bacteria, and improper lighting may render the result slightly in doubt. It is to eliminate this possibility that this modified technic is suggested.

The sputum is collected from the patient in a sterile Petri dish and sent to the laboratory. A small amount of sputum is now transferred to a slide and stained with Gram's stain. If the pneumococci are present proceed immediately with the typing. A series of slides are laid out and numbered according to the type of serum to be used. On each of these slides is placed a small drop of sputum. To this sputum is added one or two drops of the particular serum to be used. This is thoroughly mixed and each slide allowed to remain in a small cabinet away from dust for thirty minutes. The slides are then removed, fixed with heat, and stained for one minute with methylene blue, washed and dried under the oil immersion lens. If the pneumococci are present and are of one

*When the amount of the color obtained is dark, dilution may be increased to 50 or 100 c.c.; however, when the amount of protein is very great it is best to repeat the determination using 0.5 c.c. of spinal fluid.

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of the types tested the typical Neufeld capsular swelling is immediately apparent, and the specimens are so vivid the chances of error are completely eradicated.

CONCLUSION

A modified Neufeld test is described, using the fixed and stained preparation. The marked contrast between the living and fixed preparations is so outstanding as to make an error almost impossible.

Appreciation is expressed to Mrs. Dave Regan, Chief Technician of the Middletown Hospital, for her technical assistance.

SOME PRACTICAL ASPECTS OF THE ROUTINE PERFORMANCE OF THE KAHN TEST*

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THE Kahn test has found a most important place in the laboratory diagnosis of syphilis, either used alone or in conjunction with one or more serologic tests. Of thirty laboratories participating in a recent survey conducted by the United States Public Health service,[1] twelve performed the Kahn standard flocculation test. This number was exceeded only by those performing the Kolmer complement-fixation test. Since the Kahn test has become so generally and favorably known it has been widely adopted for use in many of the smaller, as well as the larger laboratories. Very often it is the only test for syphilis which is employed in any one laboratory. The test warrants its popularity because it satisfies in a relatively high degree the criteria for an efficient serodiagnostic test, namely, sensitivity and specificity, ease in reading the results, and the rapidity and economy of performance.[2]

The description of the technique of the Kahn test is available in detail to everyone.[3] In this laboratory the Kahn standard test has been performed routinely along with a complement fixation test since July, 1929. During this period approximately 273,824 specimens have been examined. In the routine examination of this large number of specimens, we have become aware of several factors of practical importance in the performance of the test, concerning which there is little or no reference made in the literature. Since the test is so widely used, a discussion of the difficulties encountered is of especial concern to technicians.

These factors herein discussed are:

1. The advisability of performing the Kahn presumptive test in addition to the Kahn standard even though a complement fixation test is employed.[4]
2. Anomalous reactions in which the 3:1 serum to antigen-suspension ratio is strongly positive and the greater antigen dilutions are weakly positive or negative.[5]
3. Serums giving strongly positive complement fixation reactions, accompanied by a negative Kahn, the latter becoming strongly positive upon dilution.[3]
4. Effect of temperature on the tests.

*From the State Hygienic Laboratories.
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THE PRESUMPTIVE KAHN TEST

The presumptive Kahn test employs a more sensitive antigen than that used in the standard test. Because of its higher sensitivity it was not intended as a diagnostic procedure. When the Kahn standard test is the only one employed in the diagnosis of syphilis, the presumptive is recommended as a check on the results of the standard test. When a second method, as one of a complement fixation type, is used in conjunction with the standard Kahn test, it is generally deemed unnecessary to include the presumptive, the second test acting as a check upon the first. We feel, however, that the inclusion of the Kahn presumptive test is of definite value even though the standard Kahn and a complement fixation test are already employed.

Because of the high sensitivity of the presumptive test, a negative reaction has a greater significance than a similar reaction given with less sensitive methods.[4] On the other hand a positive reaction with two tests and confirmed by that of a third, checks laboratory technique and gives almost certain proof of syphilitic infection. Because the presumptive test is more sensitive, positive reactions persist longer in cases under treatment, thus serving as an additional guide to the progress and effectiveness of treatment.[4] Our experience has confirmed the observations of Cajigas[4] that the Kahn presumptive reaction has been positive in known syphilitic cases under treatment, in which the complement fixation and standard Kahn tests gave completely negative results.

In this laboratory we have performed the standard 3 tube Kahn and a complement fixation test routinely on every specimen (quantity of serum permitting). The source of the serums examined is from the service to the physicians of Iowa, the State University Hospitals, and the State mental and penal institutions. Thus, undoubtedly all types of syphilis are represented.

During 1936 the Kahn presumptive test was included as a routine procedure on all specimens. Of the 19,722 serums examined, 2,642 gave some reaction with either or both the standard Kahn and the complement fixation tests. Of this number 153 gave a positive presumptive, a negative standard Kahn, and a positive complement fixation reaction. The clinical importance of these positive presumptives is indicated by the presence of a positive complement fixation reaction in each instance. Thus if the standard Kahn test had been used alone, no serologic evidence of syphilis would have been found in 5.8 per cent of the reacting serums. Likewise if the presumptive test had not been performed there would have been no confirmation of the positive complement fixation reaction.

Due to the nature of the specimens received in a public health laboratory, we feel that any additional information given the physician in even a small number of cases is worth while. Since the great majority of reports are sent to general practitioners who under the circumstances must arrive at a diagnosis and arrange therapeutic programs based largely on the reports obtained from the laboratory,[6] we feel that the inclusion of the supplementary test is warranted.

ANOMALOUS REACTIONS

When the Kahn test was devised, the optimum range of serum to antigen-suspension ratios was found to be 3:1, 6:1, and 12:1 corresponding to the front,

middle, and back tubes respectively, of the standard test. Tested against known positive serums, Kahn found that these ratios gave the largest percentage of reactions. The routine examination in this laboratory of 30,842 serums, showed 2,954 typical Kahn positive reactions, i.e., each tube showed a strongly positive reaction, or the amount of precipitate increased from the front to the back tubes. For example serums showing precipitates as 4 4 4, 3 4 4, 2 4 4 are typical positive reactions. Not infrequently there are reactions such as 4 4 3, 4 3 1, 4 4 –, which may be safely interpreted as strongly positive. Less often, reactions such as 4 1 –, 4 – –, are obtained. Kahn[3] recommends dilution of these serums, and if each tube shows a definite precipitate the final reaction is interpreted as four-plus. Of the 30,842 serums, there were 268 which gave very atypical Kahn reactions with a 4-plus complement fixation reaction. Of this number 76 strongly positive serums gave readings such as 4 1 –, 4 – –. Upon dilution with saline all of these serums showed a zone in which there were typical 4 4 4 reactions. In every case these serums gave strongly positive complement fixation reactions. Anomalous reactions may be due in part to an inherent characteristic of the antigen used, rather than wholly to a peculiarity of the serum. However, all antigens, even though properly prepared and standardized, give a certain number of this type of reaction. In small laboratories where the Kahn test is the sole method used, these reactions though infrequent, might be perplexing to the inexperienced technician.

Table I shows ten individual reactions of this nature picked at random from our records. The results of the corresponding presumptive readings are included, showing the effect of dilution upon them also.

DECEPTIVE NEGATIVE AND QUESTIONABLE KAHN REACTIONS

We have considered the type of reaction in which the front tube, the 3:1 serum to antigen-suspension ratio, at least, shows a strongly positive precipitate. This sort of reaction would indicate evidence of a syphilitic infection and certainly would not be reported as negative. However, a certain number of serums are found which give little or no precipitate in any tube of the standard test, but at the same time giving strongly positive complement fixation reactions. It is outside the purpose of this discussion to reconcile all of the discrepant reactions obtained by any two or more serodiagnostic tests for syphilis. It is possible, nevertheless, to eliminate some of these apparent conflicting results.

In this series 30,842 specimens were examined. Each serum, showing a positive complement fixation reaction and a negative or questionably negative standard Kahn reaction, was diluted with saline. A standard Kahn test was then performed on each dilution.

Table II shows a group of reactions of this nature taken at random from our records. The results of the respective presumptive readings are included showing the effect of dilution upon them.

According to Levine[7] this phenomenon may be explained by the theory that in some positive cases the optimum quantitative relationship between the oppositely charged reacting colloids may not be obtained in any one or all of the antigen ratios used in the Kahn test.

TABLE I

SPECIMEN NO.	STANDARD THREE TUBE KAHN TEST															COMPLEMENT FIXATION TEST	KAHN PRESUMPTIVE				
	UNDILUTED			1:2 DIL.			1:5 DIL.			1:10 DIL.			1:20 DIL.				UNDIL. SERUMS	DILUTIONS			
	3:1	6:1	12:1	3:1	6:1	12:1	3:1	6:1	12:1	3:1	6:1	12:1	3:1	6:1	12:1			1:2	1:5	1:10	1:20
1	4	±	–		*		4	4	4	–	4	4	–	–	1	4+	–	4	4		
2	4	±	±				4	4	4	4	4	4	–	2	4	4+	2	4	4		
3	4	1	1				4	4	4	±	4	4				4+	–		4		
4	4	±	±				–	4	4	–	3	4				4+	–		4		
5	4	±	–	4	±	±	4	4	4	4	4	4	–	±	3	4+	–	–	4	4	4
6	4	–	–				4	4	4	–	4	4	–	–	±	4+	–	4	4	4	–
7	4	–	–	4	4	–	4	4	4	–	–	3				4+	–	4	–		
8	4	1	1	4	4	4	4	4	4	1	4	4	–	3	4	4+	1	4	4	4	
9	4	1	±				4	4	4	–	4	4				4+	1	4	4		
10	4	1	–	1	–	–	4	4	4	1	4	4	–	4	4	4+	–	2	4	4	

*Blank spaces indicate there was insufficient serum to permit these dilution ratios.

TABLE II

SPEC. NO.	STANDARD THREE TUBE KAHN TEST															COMPLEMENT FIXATION REACTION	UNDIL. SERUMS	KAHN PRESUMPTIVE			
	UNDILUTED			1:2 DIL.			1:5 DIL.			1:10 DIL.			1:20 DIL.					1:2	1:5	1:10	1:20
	3:1	6:1	12:1	3:1	6:1	12:1	3:1	6:1	12:1	3:1	6:1	12:1	3:1	6:1	12:1						
1	±	±	±	4	3	3	4	4	2	4	4	4	4	4	4	4+	–	4	4	4	4
2	±	±	±		*		4	4	1	4	4	4	4	4	4	4+	–	4	4	4	4
3	±	±	±							4	4	±	4	4	4	4+	–		–	4	4
4	±	±	–	4	–	–	4	±	–	4	4	4	–	4	4	4+	–	4	4	4	4
5	±	±	±	4	4	2	4	4	4	–	4	4				4+	–	4	4		
6	–	–	–				4	4	4	4	4	4				4+	–	4	4		
7	–	–	–	4	–	–	4	4	4	3	4	4				4+	–	1	4		
8	±	±	–	4	±	–	4	4	±	4	4	4	2	4	4	4+	–	–	4		
9	1	±	–				4	4	–	4	4	4				4+	1+	4	4	4	
10	±	±	±	4	4	±	4	4	4	–	±	4	–	–	–	4+	–	1	4	4	

*Blank spaces indicate there was insufficient serum to permit these dilution ratios.

Since the concentration of reagin as evidenced by precipitation is not constant in all serums, a range of dilutions was made in this procedure.

In this group only two serums were marked for control of treatment, the balance for diagnosis. Granting that the latter group may have included individuals who had received treatment, it may not be assumed that these reactions are particularly incidental to treatment. They have been found in heretofore undiagnosed cases of long standing.

This anomaly may seem of little importance because of its infrequent occurrence. In laboratories performing at least two serodiagnostic tests for syphilis some of the apparent contradictory reactions on one specimen can be avoided by this dilution procedure. In laboratories where the standard Kahn test is the sole serodiagnostic method employed, obviously a negative report might be given to a clinician who could not correlate it with other positive findings or what would be more significant, dismiss the case with no diagnosis of syphilis being made in the presence of all other negative clinical symptoms. Under certain circumstances reports such as these might result in adverse criticism and lack of faith in an individual technician or laboratory.

In order to establish a certain degree of confidence, from the standpoint of both the laboratory and the physician, and to best serve the serology of syphilis, it is definitely advisable to perform more than one serodiagnostic test. Preferably a reliable modification of the Wassermann reaction possessing the maximum of sensitiveness consistent with specificity[6] should be employed with a carefully controlled, titrated test as is the Kahn precipitation test. A complement fixation test may give information not obtainable by a precipitation test. On the other hand a precipitation test may be positive when the complement fixation test is negative.[2]

EFFECT OF CLIMATIC CONDITIONS ON THE TESTS

Temperature is known to have an effect on the Kahn test. In our experience with large numbers of tests which we perform routinely, the temperature of the room in which the tests are performed has a definite relationship to the accuracy and ease with which the tests are read. At low temperatures, due to the season of the year, drafts, or practical working facilities, the precipitates in the positive reactions are insufficiently developed so that they are difficult to read. Even the negative reactions are difficult to distinguish from weakly positive ones. After an incubation period of ten minutes at 37° C. before shaking, the precipitates become heavier and better formed. This also clarifies the negative reactions. This method has not been generally advised, however, because of the danger of obtaining nonspecific weak reactions due to an increased sensitivity.

With reference to this factor of climatic effect, a record of atmospheric conditions was kept over a period of eight months. The data included temperature, relative humidity, barometric pressure, whether the day was clear or cloudy, and whether the Kahn tests were easy or difficult to read. A constant antigen titer and identical technique, exclusive of the individual personal factor, were employed each day. As might be expected the tests were somewhat easier to

read on bright days. No other condition seemed to have any constant bearing on the readability of the tests except that of temperature. Relative humidity, or barometric pressure had no appreciable effect. When the temperature of the room dropped below 75° F., there were more cloudy or fuzzy reactions than there were above that temperature. These indefinite reactions in addition to being difficult of evaluation present a definite strain and tension on the part of those reading the tests. We have tried, therefore, as nearly as possible to avoid low temperatures or drafts on the reagents while actually performing the tests, and we have had uniformly better results.

CONCLUSIONS

1. The presumptive Kahn test should supplement the standard three tube Kahn test even though a second serodiagnostic test, such as a complement fixation test, is routinely employed.

2. Serums which give very anomalous reactions may demonstrate a definite positive zone upon dilution with saline.

3. Certain serums which give a negative Kahn reaction, may also show a positive zone phenomenon upon dilution, thus confirming the presence of a strongly positive complement fixation reaction.

4. Temperature affects the readability of the tests, the higher temperatures giving better results.

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AN IMPROVED METHOD FOR THE DETERMINATION OF URINE UREA NITROGEN BY DIRECT NESSLERIZATION*

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EVER since the introduction of the blood urea clearance estimation as an early index of diminution of kidney function, efforts have been made by clinicians and laboratory workers to simplify the procedures necessary for the determination of blood and urine urea nitrogen. In our hands the Taylor-Blair[1] method has proved satisfactory for the determination of blood urea nitrogen, both from the standpoint of speed and accuracy. This method has been commented upon by Shapiro,[2] Eveleth,[3] and others, who have found the method reliable for clinical use.

While the aeration method of Van Slyke and Cullen[4-6] has proved quite satisfactory from the standpoint of accuracy in the determination of blood and urine urea nitrogen, there are many objections to it from the standpoint of speed and simplicity of manipulation. Kay and Sheehan[7] have recently pointed out some of the precautions which are necessary for accuracy in the use of this method. They have also called attention to the fact that, while the rate of aeration is not the factor which determines the removal of the ammonia from the reaction tube, it is impossible to remove more than 96 per cent of the ammonia from the reaction tube to the acid tube in two hours without producing alkaline misting. All of these factors contribute to make the Van Slyke and Cullen method rather unsatisfactory for routine use in the clinical laboratory.

Marshall's[8] titration method, while somewhat simpler, is still slow, and in our hands has not given as satisfactory results as the aeration method.

In the determination of urine urea nitrogen, as in the determination of blood urea nitrogen, the method of choice, from the standpoint of speed and ease of manipulation for clinical work, would be one which employed direct nesslerization. The outstanding objection to all direct nesslerization procedures is the turbidity which often developes before a comparison can be made colorimetrically with the standard solution. We believe that by applying the principle of the Taylor-Blair method to diluted urine we have been successful in overcoming this obstacle.

METHOD

If the urine specimen is neutral or alkaline, render it just acid to litmus with glacial acetic acid; then transfer exactly 1 c.c. of the urine to a 100 c.c. volumetric flask and make up to the mark with distilled water. Place about

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25 c.c. of this diluted urine in a 125 c.c. Erlenmeyer flask and add 1 or 2 gm. of permutit; shake gently with rotating motion for five minutes. Filter to remove the permutit. Pipette 10 c.c. of the filtrate into another 125 c.c. Erlenmeyer flask. Add 10 c.c. of distilled water and two drops of the special urease reagent. Stopper and incubate at 50° C. for fifteen minutes. Now add 20 c.c. of water. To this add 5 c.c. of 10 per cent sodium tungstate solution and 5 c.c. of ⅔ N sulphuric acid. Shake gently for two minutes and filter through a good grade retentive filter paper, such as Whatman No. 42. To 10 c.c. of this filtrate in a 50 c.c. volumetric flask add about 25 c.c. of distilled water and 5 c.c. of Nessler's solution. Make up to the mark with distilled water.

At the same time prepare a standard. Place 3 c.c. of the standard ammonium sulphate solution (1 c.c. contains 0.1 mg. of nitrogen) in a 100 c.c. volumetric flask, add about 75 c.c. of water and 10 c.c. of Nessler's reagent and make up to the mark with water.

Compare the standard and specimen in a colorimeter and compute the percentage of urea nitrogen by the following equation.

$$\frac{R_s}{R_u} \times 0.3 \times \frac{100}{0.02} \times \frac{50}{100} \times \frac{1}{1000} = \text{per cent of urea nitrogen.}$$

R_s and R_u are the colorimetric readings of the standard and specimen, respectively.

If the colors do not match within rather narrow limits in the colorimeter, a smaller or larger amount of the filtrate may be used in the nesslerization, and a suitable correction for the quantity of urine made in the formula.

REAGENTS

1. Special urease reagent.
2. Permutit.
3. 10 per cent sodium tungstate solution.
4. ⅔ N sulphuric acid.
5. Standard ammonium sulphate solution, each c.c. of which contains 0.1 mg. of nitrogen.
6. Nessler's reagent.

PREPARATION OF THE SPECIAL UREASE REAGENT[1]

"Shake 75 gm. of 'Arlco' (defatted) jack bean meal with 400 c.c. of a 32 per cent acetone solution at 28° C. for five minutes. Filter through a fluted filter paper in the refrigerator. Filtration is rapid and the filtrate should come through clear. Add 15 c.c. of N/10 acetic acid to the acetone filtrate and allow to remain on ice overnight. Centrifuge and pour off mother liquor. Wash precipitated urease twice by stirring up with ice cold 32 per cent acetone-phosphate solution, made by diluting c.p. acetone to 32 per cent with a phosphate mixture containing 143 c.c. of 0.1 M NaOH and 500 c.c. of 0.2 M KH_2PO_4, and centrifuging. As pointed out by Sumner, the iso-electric point of urease is somewhere in the neighborhood of pH 6.1, and the precipitated urease is least soluble in the acetone-phosphate solution mentioned above.

Pour off the acetone-phosphate mixture and drain tubes against filter paper until free from the odor of acetone. Tubes may be placed in the incubator at 37.5° C. for a few minutes to free from acetone, if care is exercised not to let the precipitated urease dry, which rapidly inactivates it. Unlike the crude urease preparations, it cannot be dried on paper without complete inactivation. Suspend the washed precipitate of urease in 5 c.c. of ammonia-free distilled water. Add 0.1 c.c. of toluene containing 5 per cent of thymol as a preservative and keep tightly stoppered on ice in a small bottle. The preparation will keep on ice for at least a month, and in our hands such preparations have developed only a negligible amount of ammonia. The washed sediment will contain the urease in a highly concentrated form (globulin?), and should not give a test for ammonia nitrogen or free carbohydrate."

EXPERIMENTAL

Experiments will be cited to show the results obtained by direct nesslerization in comparison to those obtained by aeration and direct titration; also the results of recovery experiments using the direct nesslerization method will be shown.

1. The urea nitrogen content of a series of urines was determined by using the aeration, direct titration, and direct nesslerization methods. The results are shown in Table I.

TABLE I

SHOWING THAT WITH THE IMPROVED METHOD OF DIRECT NESSLERIZATION OF FILTRATE FROM UREASE TREATED URINE ONE OBTAINS APPROXIMATELY THE SAME URINE UREA NITROGEN VALUES AS IN THE AERATION AND TITRATION METHODS

(Results are expressed as gm. urea nitrogen per 100 c.c. of urine)

URINE NO.	DIRECT TITRATION	AERATION	DIRECT NESSLERIZATION
1	0.579	0.482	0.491
2	0.785	0.706	0.720
3	0.636	0.560	0.552
4	1.070	0.996	1.097
5	0.491	0.477	0.575
6	0.677	0.627	0.664
7	1.247	1.135	1.210
8	0.635	0.612	0.584
9	0.374	0.397	0.383
10	0.331	0.336	0.331
11	0.402	0.407	0.384
12	0.196	0.192	0.201

2. Known quantities of urea were added to samples of urine and the urea recovered using the direct nesslerization method. Results are shown in Table II.

TABLE II

SHOWING THE RECOVERY OF ADDED UREA BY THE IMPROVED METHOD OF DIRECT NESSLERIZATION

(Results expressed as gm. of urea nitrogen per 100 c.c.)

URINE UREA N	UREA N ADDED	TOTAL UREA N PRESENT	UREA N FOUND	UREA RECOVERED PER CENT
0.571	0.568	1.139	1.137	99.7
0.491	0.502	0.993	1.000	100.7
0.313	0.233	0.546	0.547	100.2
0.682	0.369	1.051	0.940	89.3
0.570	0.568	1.138	1.125	98.8
0.376	0.626	1.002	1.015	101.3

3. Blood urea clearance determinations were done on thirty-two medical students using the Taylor-Blair method for the determination of blood urea nitrogen and the proposed direct nesslerization method for the determination of the urine urea nitrogen. None of these students showed abnormal constituents in their urine by chemical examination, and in all cases the blood urea clearance values were well within the accepted range of normal.

SUMMARY

1. A new method for the determination of urea nitrogen in urine has been developed. The urine, if neutral or alkaline, is made just acid to litmus with glacial acetic acid, diluted, and treated with permutit to remove the ammonia. The urea in the urine is then hydrolyzed to ammonium carbonate by the action of a highly concentrated urease reagent in fifteen minutes at 50° C. The concentrated urease and any other interfering proteins are then removed from the urine by treatment with tungstic acid. Urea nitrogen is determined colorimetrically, without turbidity interference, after nesslerization of the filtrate.

2. The above method for the determination of urine urea nitrogen is an application of the Taylor-Blair method for determining blood urea nitrogen.

3. In our hands this method for determining urine urea nitrogen values has proved comparable in accuracy to the standard aeration and titration methods. It has shown a high degree of accuracy in the recovery of urea added to urine.

4. The method offers a degree of ease and practicability of manipulation which allows many determinations to be made at the same time, requires no additional apparatus, and eliminates technical difficulties and inaccuracies of aeration and distillation procedures.

5. This method, when combined with the Taylor-Blair method for the determination of blood urea nitrogen, gives a marked degree of speed and accuracy, which makes the blood urea clearance determination as a clinical measure of kidney function readily available.

NOTE: We wish to express our thanks to Dr. J. M. Hill, Director of Baylor Hospital Laboratories, for his assistance in making urine specimens available for this work, as well as to Miss M. M. Craver for her technical assistance.

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THE RAPID PREPARATION OF TAPEWORM PROGLOTTIDS FOR DIAGNOSTIC AND TEACHING PURPOSES*

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I. INTRODUCTION

THE method to be described is the result of many attempts to secure a tapeworm preparation which could at the same time be used practically for diagnosis and as a permanent museum specimen. Our aim was to devise a staining method which would define the outlines of the uterus of the tapeworm proglottid. Since the ova determine the outlines of the uterus, a plausible method would be one which would insure staining of the ova and would render the remainder of the tissue transparent. For this purpose fresh material from human and animal sources was employed; namely, *Taenia saginata* and *Taenia marginata*.

II. PROCEDURE

1. Sections measuring 4 or 5 cm. in length were cut from that portion of the tapeworm presenting gravid proglottids. These sections were placed between glass slides and held in place with elastic bands. They were then immersed in a modified Bouin's solution (70 per cent saturated aqueous picric acid, 20 per cent glacial acetic acid, 10 per cent formalin) where they remained for ten to twelve hours.

2. The sections were washed in running tap water for two or three minutes.

3. The sections were placed horizontally and flooded with 10 per cent NaOH. Changes in the tissue were closely observed. An orange color was noted to diffuse into the hydroxide solution. There was a general decolorization peripherally. At the same time, centrally, the outlines of the uterus became visible as a deep orange.

4. The preparation was then rinsed in tap water.

5. The preparation was flooded with 5 per cent HCl for one or two minutes.

6. It was then returned to the tap water-bath where it was left for 10 or 15 minutes.

7. The preparation was then advanced through the alcohols (30, 50, 70, 85, and 95 per cent) allowing four or five minutes in each chamber.

8. Xylene was used in clearing, complete clearing requiring from 7 to 10 minutes.

9. The preparation was then mounted with a cover glass in gum dammar.

The completed preparation showed orange-stained ova outlining the uterus; the remainder of the tissue was pale yellow to colorless.

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III. RATIONALE

When the tissue is placed in the modified Bouin's solution, there is a combination of the protein of the tissue with the picric acid. This solution, by virtue of its relatively higher glacial acetic acid content (more than four times that of the ordinary Bouin's solution), affords better penetration of the tissues by the picric acid. This is desirable since the ova are then better stained and the proper staining of the ova is fundamental to this method. The combination of the protein of the tissue with the picric acid, a protein picrate, is insoluble in weak acids and alcohol. When the 10 per cent NaOH is added, a soluble compound forms which can be washed from the tissues. The new soluble compound is sodium picrate, which is orange in color. As the sodium hydroxide penetrates, the orange sodium picrate, as it forms, diffuses from the superficial portions of the tissues, but leaves the deeper portions less readily. The ova become visible by their orange staining. Before this staining can be lost by diffusion, the process is stopped by immersing the preparation in the 5 per cent HCl solution.

Probable Reactions.—The protein of the tissue, as any other protein, may act as an amphoteric electrolyte. Each protein has its characteristic isoelectric point, at the pH of which, the protein molecule bears no charge. If the medium be made more acid, the pH lowered, the protein molecule acquires a positive charge; likewise, addition of alkali to the isoelectric protein causes formation of a salt of the metallic ion and the protein molecule, the latter now acting as a negative ion. The reactions, as they apply here, are presented below. Only the picric acid of the acid components is considered since it is the staining agent.

$$\text{protein picrate} \xleftarrow[\text{acid}]{\text{picric}} \text{isoelectric protein} \xrightarrow[\text{hydroxide}]{\text{sodium}} \text{sodium proteinate}$$

Addition of the 10 per cent NaOH produces a change in the color of the tissue, namely, from yellow, due to the protein picrate, to orange, due to the newly formed sodium picrate. Also, the change is from an insoluble to a water-soluble picrate compound.

$$\text{protein picrate (yellow and water-insoluble)} \xrightarrow[\text{sodium hydroxide}]{} \text{sodium picrate, sodium proteinate}$$

The basis for this reaction is the inability of the protein molecule to act as a positive ion in a strongly alkaline solution; it can no longer hold the picrate radical in combination, sodium picrate forming consequently. Moreover, because the medium is on the alkaline side of the isoelectric point of the protein, the protein molecule must assume a negative charge; it combines with the readily avaliable sodium ions. The differentiation of the preparation depends upon the selective removal of the soluble sodium picrate.

IV. SUMMARY

A simple method for the preparation of tapeworm proglottids is described. This method, which requires a minimum in materials readily available, has given consistently good results in our laboratory. An explanation of the staining reaction, in chemical terms, is suggested.

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A COMPARISON OF UREA METHODS*

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THERE are numerous methods in use for the determination of urea in blood, and since the urea content of the blood has clinical significance, the question often asked is "which of these methods is it best for the clinician to use?" Methods have been compared as to accuracy and simplicity, but it appeared that a more comprehensive comparison, including the methods which represent the various principles for urea determination, would be desirable.

For this work the methods chosen are in common use and representative of the various principles, but the number of methods were limited so that all the analyses could be made on the same sample of blood during one day.

A 10 per cent solution of urease prepared from Squibb's urease tablets was used in all the urease methods, except in the Van Slyke and Plazin method when the urease tablet was crushed and added directly to the blood. For nesslerization the Koch and McMeeken Nessler's solution (2) was used.

The following methods were used:

Van Slyke and Cullen.[1]—Three cubic centimeters of blood, 3 c.c. of phosphate buffer, 5 drops of caprylic alcohol, and 1 c.c. of urease solution were digested in a water-bath at 50° C. for fifteen minutes, after which 10 c.c. of saturated potassium carbonate solution were added and the ammonia aerated into 25 c.c. of 0.01 N acid. The excess acid was titrated with 0.01 N sodium hydroxide using methyl red as an indicator.

Van Slyke and Plazin.[2]—A crushed tablet of Squibb's urease was added to 5 c.c. of blood and digested at 50° for ten minutes. A zinc hydroxide filtrate according to Somogyi[3] was prepared. To 5 c.c. of the filtrate were added 15 c.c. of water and 5 c.c. of Nessler's solution, and this solution was compared with a standard ammonia sulphate solution, similarly treated.

Folin and Wu.[4]—Two-tenths cubic centimeter of urease solution and 0.15 c.c. of buffer solution were added to 5 c.c. of Folin-Wu filtrate, and the mixture was digested for ten minutes at 50° C. After digestion, 2 c.c. of saturated borax solution were added and the ammonia distilled into acid by means of a micro-Kjeldahl apparatus. This was followed by nesslerization and comparison with a nitrogen standard.

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Karr.[5]—To 5 c.c. of Folin-Wu filtrate were added 5 drops of urease solution and 5 drops of buffer solution. This was digested for ten minutes at 50° C., after which the solution was nesslerized and compared with a standard ammonium sulphate solution, similarly treated.

Keller's Micromethod.[6]—Two-tenths cubic centimeter of blood were added to 9.8 c.c. of tungstic acid solution, the precipitate centrifuged out, and 4 c.c. of the supernatant fluid treated with 3 drops of urease solution and 3 drops of phosphate buffer solution. A standard urea solution was treated in like manner and both solutions placed in a water-bath at 50° C. for ten minutes. Both solutions were diluted to 9 c.c.; 1 c.c. of Nessler's solution was added, after which they were compared in a colorimeter.

Van Slyke's Manometric Urease Method.[7]—Procedure B, in which carbon dioxide is liberated and measured, was used. Two modifications were made; first, the urease solution and the phosphate buffer solution were added separately by means of rubber tipped pipettes (phosphate solutions destroy or weaken the urease solution if allowed to stand together for any length of time). Second, five minutes were allowed for the urease to react with the urea.

Beattie's Xanthydrol Colorimetric Urea Method.[8]—Two molecules of xanthydrol combine with one molecule of urea to form xanthydrol ureide. The test was made on 1 c.c. of Folin-Wu filtrate. The precipitate of xanthydrol ureide was collected in a Gooch crucible packed with asbestos and washed with methyl alcohol and water. It was then dissolved and made up to volume with 50 per cent sulphuric acid. This was compared in a colorimeter with a similar solution prepared from a standard urea solution.

Van Slyke and Kugel Hypobromite Urea Method.[9]—Five cubic centimeters of Somogyi's zinc hydroxide blood filtrate were treated in the reaction chamber of the manometric apparatus with the sodium hypobromite mixture. The pressure of the freed nitrogen was measured and the urea calculated from tables.

The Autoclave Hydrolysis Method of Folin and Wu.[4]—When urea is heated to 155 to 255° C. in acid or alkali, it decomposes, forming ammonia and carbon dioxide. To test tubes were added, respectively, 5 c.c. of Folin-Wu blood filtrate and a standard urea solution. To each was added 1 c.c. of N sulphuric acid. The mouths of the tubes were closed with tin foil, after which they were autoclaved at 150° C. for ten minutes. The contents of each were placed in 100 c.c. cylinders, nesslerized, and compared in a colorimeter.

Titration of Urea With Mercuric Salts.—The special apparatus designed by the LaMotte Company, which makes use of the Hench and Aldrich method,[10] was used. A trichloracetic acid filtrate of blood is titrated with mercuric chloride. Mercuric chloride reacts with urea and also with creatinine, creatine, and uric acid to form a complex salt. When an excess of mercuric chloride has been added, a drop of the filtrate added to a drop of sodium carbonate solution forms a brown precipitate.

DISCUSSION

A series of ten analyses were made by each procedure on samples of cow blood. The Van Slyke manometric urease method showed the least maximum variation, which was 0.35 mg. of urea nitrogen per 100 c.c. of blood. The

other methods had variations of 0.43 mg. for the autoclave hydrolysis method; 0.5 mg. for aeration titration; 0.61 mg. for the Van Slyke and Kugel hypobromite method; 0.7 mg. for the xanthydrol colorimetric method; 0.8 mg. for the Van Slyke-Plazin method and the Keller micro-urease method; 0.9 mg. for Karr's direct nesslerization of Folin-Wu filtrate; 1.1 mg. for the Folin-Wu urease method with distillation; and 2.8 mg. for the LaMotte procedure.

Varying amounts (5 mg. to 100 mg.) of urea nitrogen per 100 c.c. of blood were added to cow blood. The recoveries for each method were similar to those cited above.

Samples of blood were then analyzed by each of the ten methods so that a comparison of the results might be made. Table I presents data on samples of cow blood. When extra urea was added, the amount of urea present in the blood was determined by the Van Slyke manometric urease method.

Table II includes determinations made on blood from 10 normal human subjects and Table III, data on 10 pathologic subjects in whom the urea nitrogen ranged from normal to 250 mg. of urea nitrogen per 100 c.c. of blood.

From this study it appears that the Van Slyke manometric urease method is the most accurate, since it gave the least variation on blood filtrates and showed the best recovery on samples of blood to which urea had been added. However, the method has certain disadvantages for routine use in hospitals, clinics, and private practice. First, the cost of the manometric apparatus is rather high; second, it requires considerable technical skill in order to obtain accurate determinations; third, when one makes only an occasional determination it requires a more rigid inspection of the apparatus than is required of most other urea methods. Special attention should be called to the necessity of adding the *urease* and *phosphate buffer* separately, and allowing sufficient time for the reaction; otherwise the values may be as much as 50 per cent low.

The values obtained by the aeration and titration method closely parallel those of the above method, the greatest variation being 2.1 mg. on a sample with over 250 mg. per 100 c.c. The apparatus required is inexpensive. The disadvantages of the procedure are: 2 or 3 c.c. of blood are required and almost two hours are needed for an analysis. However, when several determinations are being made and other experiments can be carried out during the aeration period, this time factor is negligible. Also, enough standard acid is used so that any range from normal to the highest values can be determined correctly.

Karr's method of direct nesslerization of the Folin-Wu filtrate gave results which varied from the manometric urease method values by −2.1 mg. to +7.7 mg. urea nitrogen. The greatest variations were found in pathologic blood with high urea content. Like all nesslerization methods there is a tendency for the unknown to be turbid unless the value is in a limited range. If turbidity occurs it is necessary to repeat the test, using a smaller amount of blood filtrate. Also, the added urease tends to give varying shades of color which increase the difficulty of comparison. The Folin-Wu method, in which distillation proceeded in nesslerization gave results lower than the manometric procedure in all except two determinations. The maximum variation was −5.4

TABLE 1

VARIATIONS IN THE AMOUNT OF UREA NITROGEN BY THE VARIOUS METHODS USING COW'S BLOOD

METHOD	1	2	3	4	5	6	7	8	9	10
Mg. urea nitrogen per 100 c.c. blood					16.9	16.88	16.05	17.2	15.1	16.1
Mg. urea nitrogen added per 100 c.c. blood					5	10	15	20	50	100
Total mg. urea nitrogen per 100 c.c. blood					21.9	26.88	31.05	37.2	65.1	116.1
Aeration and titration	16.07	16.1	16.4	15.1	21.92	26.8	31.4	38.3	64.3	115.22
Method by Van Slyke and Plazin	15.79	15.66	16.3	15.5	20.8	26.6	31.2	38.04	65	114.3
Folin and Wu urease method with distillation and nesslerization	14.6	14.9	15.9	15.1	19.6	25.2	28.7	40.4	62.3	111.8
Folin and Wu filtrate and direct nesslerization by Karr	16.8	15.4	17.4	14.2	22.1	26.1	30.6	39.3	65.2	113.6
Keller microurease method	15.62	15.4	17.1	16.2	19.7	24.7	30.1	34.7	61.6	112.8
Van Slyke manometric urease	15.96	16.09	16.56	15.32	21.84	26.978	31.1	37.77	64.88	115.74
Van Slyke and Kugel hypobromite	16.6	15.67	16.38	15.47	22.3	27.2	31.85	37.9	64.9	116.36
Autoclave hydrolysis	16.2	16.66	17.2	15.9	22.4	27.4	32.4	41.6	66.3	117.1
LaMotte	16.82	14.02	17.8	13.7	21.08	24.3	30.36	42	63.1	115.6

TABLE II

COMPARATIVE UREA VALUES OBTAINED BY THE VARIOUS METHODS ON NORMAL BLOOD*

METHOD	S	R	B	ST	N	W	O	OL	D	NJ
Aeration and titration	14.6	13.8	13.6	15.1	14.9	14.3	14.7	16.2	13.3	14.1
Van Slyke and Plazin	14.6	13.8	13.8	15.3	14.6	14.2	14.4	16.4	13.2	14.0
Folin and Wu urease method with distillation and nesslerization	14.4	13.1	13.3	15.6	14.6	14.4	14.4	15.8	12.8	14.1
Folin and Wu filtrate and direct nesslerization by Karr	16.1	12.9	14.9	13.4	15.3	14.8	15.2	15.9	14.1	14.2
Keller micromethod	15.6	11.4	15.2	15.7	14.4	15.6	13.7	15.4	14.0	14.6
Van Slyke manometric	14.7	13.62	13.62	15.31	14.84	14.44	14.52	16.12	13.09	14.21
Xanthydrol colorimetric by Beattie	15.3	13.4	13.8	14.8	14.5	13.92	14.5	15.6	13.2	14.4
Van Slyke and Kugel hypobromite	14.9	14.1	13.34	15.12	14.66	14.62	14.62	16.48	13.12	14.34
Autoclave and hydrolysis	16.3	16.1	15.2	16.1	15.7	15.2	16.1	16.4	13.8	14.4
LaMotte	16.7	15.3	12.2	17.4	14.6	12.1	16.4	16.0	14.4	15.3

*All samples of blood were collected before breakfast and potassium oxalate was used as an anticoagulant.

TABLE III

COMPARATIVE UREA VALUES OBTAINED BY THE VARIOUS METHODS ON PATHOLOGICAL BLOODS*

METHOD	OL	ND	HA	NT	CR	OS	CO	J	HE	A
Aeration and titration	16.8	20.49	26.72	36.4	69.14	97.9	94.3	171.02	191.6	249.5
Van Slyke and Plazin	16.6	21.4	26.7	36	70	96	93.6	171.4	193.4	250
Folin and Wu urease method with distillation and nesslerization	16.4	20.9	26.3	35.8	68.7	97.2	92.3	169.8	188.9	246.2
Folin and Wu filtrate and direct nesslerization by Karr	16.4	21.6	26.6	40.6	71.6	104.2	102.3	176.2	198.3	258
Keller micromethod	14.8	19.8	25.4	34.7	67.2	93.9	90.8	167.2	189.2	238
Van Slyke manometric	16.8	21	26.68	36.7	69.08	98.4	94.6	171.3	194.2	251.6
Xanthydrol colorimetric by Beattie	16.6	20.9	26.6	40.4	68.97	100.6	97.6	174	195.2	256
Van Slyke and Kugel hypobromite	17.04	20.99	26.88	40.81	69.56	101.35	100.82	172.8	196.4	252.8
Autoclave and hydrolysis	16.9	21.6	27.4	39.3	69.9	103.2	103.2	173.8	196.2	261
LaMotte	18.2	23.6	26.1	47	77.1	112	111	177.6	204.7	272.6

*Potassium oxalate was used as an anticoagulant.

mg. It appears probable that with the type of apparatus used there is a tendency for traces of ammonia to escape during the distillation.

Keller's micromethod gave values ranging from +1.58 mg. to –13.6 mg. as compared with those obtained with the manometric method.

The values obtained with the Van Slyke and Plazin method (using the zinc hydroxide filtrate) closely paralleled the Van Slyke manometric procedure, varying from +0.73 mg. to –1.6 mg. of urea nitrogen. This method was the most satisfactory colorimetric procedure since the zinc hydroxide filtrate after nesslerization did not have as great a tendency to become turbid or to differ slightly in color from the standard.

The results with the xanthydrol method compared well with the manometric method, but it required an hour or longer to perform the test, and the 50 per cent sulphuric acid in which the xanthydrol ureide was dissolved was undesirable for use in a colorimeter.

The Van Slyke hypobromite method (using the zinc hydroxide filtrate) was most satisfactory of the nonspecific urea methods. The normal values were very near those of the manometric urease method, and in the higher ranges 6.2 mg. was the greatest variation. This method has the same disadvantages as the manometric urease method, except that no urease solution is needed.

The autoclave hydrolysis method gave values which were higher than the manometric urease method, the maximum variation being +9.4 mg. with blood containing 250 mg. urea nitrogen per 100 c.c. The higher values were due to the fact that the method is not specific for urea nitrogen. The only advantage of the method is the elimination of the use of urease.

The LaMotte procedure gave the largest variations from the manometric urease method, varying from –2.67 mg. in normal bloods to +21 mg. in bloods from patients with uremia. The method is rather inaccurate but, in the authors' opinion, has a place if used for *purely clinical* purposes. These results should never be reported as urea values in the literature. Its advantages are that it requires very little technical skill, the amount of urea present in the blood sample is read directly from the special buret, the test can be performed in a short time, and it requires little laboratory space.

SUMMARY

The Van Slyke manometric urease method appears to be the most accurate method for urea determination, provided the urease is not allowed to remain in contact with the phosphate buffer except after it has been placed in the reaction chamber, and provided ample time is allowed for the reaction.

The aeration and titration method gives very accurate results, requires less technical skill than the manometric method, and, if several determinations are made at once, it is very practical. Its only disadvantage is the time required for the aeration.

The Van Slyke and Plazin method was considered the best colorimetric method as it is most accurate, is easy and quick to run, and gives less trouble with the Nessler's reagent.

The xanthydrol colorimetric method is accurate, but requires considerable time, and the use of the 50 per cent sulphuric acid solution is undesirable.

The Karr method of direct nesslerization of the Folin-Wu filtrate gave high values, while Keller's micromodification gave low values.

The Folin-Wu urease method with distillation and nesslerization gave slightly low results. It requires more time than direct nesslerization and does not improve the accuracy if the ordinary micro-Kjeldahl distillation apparatus is used.

The Van Slyke and Kugel hypobromite method gave slightly high results for specimens with high urea content, but was the most accurate of the nonspecific urea methods.

The autoclave hydrolysis method gave high urea values.

The LaMotte procedure gave varied results on normal bloods but gave high values on pathologic bloods.

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A SERUM VOLUME TEST FOR THE HEMORRHAGIC DIATHESIS IN JAUNDICE*

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THE problem of the hemorrhagic diathesis in jaundice, although it has been recognized for almost fifty years, is still unsolved. Extensive clinical observations and experimental studies have eliminated certain hypotheses, and the adoption of certain prophylactic and therapeutic measures has markedly reduced the mortality, but that is as far as we have been able to go. The cause of the accident has never been satisfactorily explained, no uniformly satisfactory test to predict it has ever been devised, and, as a corollary, the methods employed to forestall it and to control it are still without a reasoned basis and are correspondingly inadequate.

It is unnecessary to repeat here the arguments for and against the various theories which have been advanced to explain the hemorrhagic diathesis in jaundice. The facts in the case were excellently summarized by Wangensteen[1] in 1928, by Moss[2] in 1933, and by the late E. Starr Judd[3] in 1935. At present only three theories seem worthy of serious consideration, the prothrombin deficiency theory, the liver damage theory, and the defective clot theory. These we shall briefly review.

The prothrombin deficiency theory of Quick and his associates, Bancroft and Stanley-Brown[4, 5] is a by-product, as it were, of their extensive and valuable studies on thrombosis and embolism, and is based, they point out, on the concept of the coagulation of the blood held by Wohlisch. This concept includes two distinct phases: (I) Prothrombin plus thromboplastin plus calcium equals thrombin; (II) fibrinogen plus thrombin equals fibrin. The work of previous investigators, which has been confirmed by Quick and his associates, has eliminated either a calcium or a fibrinogen deficiency as responsible for the bleeding tendency. Thromboplastin also can be eliminated. Bancroft, Stanley-Brown and Kugelmass,[6] Lewisohn[7] and Nygaard[8, 9] have shown that the plasma of jaundiced patients with a hemorrhagic diathesis exhibits a prolonged clotting time on recalcification and that the abnormality is not corrected by the addition of excess thromboplastin. On the other hand, a prolonged clotting time in hemophilic patients can be promptly restored to normal by the addition of an excess of this substance. The obvious conclusion is that prothrombin is the variable element in certain jaundiced patients, and that the tendency to hemorrhage in them can be logically attributed to its presence in subnormal amounts. Quick's further conclusion that the reaction is a mass one we shall make use of later.

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The liver damage theory is based on the concept that damage to the hepatic tissue or interference with or diminution of the hepatic function may exert a deleterious effect on the coagulation of the blood. Indeed, Ivy, McNealy and their associates at Northwestern University,[10, 11] Walters[12] and others are inclined to believe that a prolonged coagulation time in jaundice is not so much an index of a bleeding tendency as it is an index of liver damage. Wangensteen has excellently summarized the experimental evidence for this theory, and it seems not illogical to assume that more or less extensive liver damage can reduce in the organism the amount of some substance, still hypothetical, which is essential for the coagulation of the blood, exactly as Mann[13] has shown that experimental hepatectomy apparently removes that substance entirely. There is still no unanimity of opinion on this point, nor any very logical speculation as to the nature of the hypothetical substance. Bancroft, Stanley-Brown and Quick are inclined to believe that the prothrombin deficiency they postulate is the result of liver damage.

Much further work is needed before the theory of liver deficiency can be accepted, and we ourselves are unwilling to assert, at this time, that hepatic dysfunction plays any very considerable rôle in the hemorrhagic diathesis. That demonstrable liver damage and a tendency to hemorrhage are sometimes associated we do not deny, but we rather question the cause and effect relationship. Our clinical observations and our experimental evidence are against it. We have seen case after case in which liver function, by such tests as are now available, was demonstrated to be seriously impaired and in which, both at operation and autopsy, the liver substance was seen to be grossly and microscopically damaged, and yet in which no bleeding occurred. In our own extensive experimental work on liver damage[14-16] we have not seen a single jaundiced animal die of hemorrhage, or even exhibit the slightest hemorrhagic tendency, either after the biliary obstruction was created or after it was released. We have handled too many dogs to be unwilling to accept this evidence, quite aside from the number of clinical cases which go to support the observation. Liver damage as an etiologic factor in the hemorrhagic diathesis perhaps cannot be discarded at this time, but surely it cannot be accepted as a fundamental cause, at least without many reservations and qualifications.

Far more logical, in our opinion, is the theory of a defect in the clot, as advanced by Ivy, McNealy and their associates, and by Carr and Foote.[17] Our own observations, which were arrived at independently, support their conclusion that the clotting mechanism in certain jaundiced patients differs from the clotting mechanism in other jaundiced patients and in normal patients. In a jaundiced individual with a hemorrhagic tendency (Fig. 1-*B*) the clot is large and bulky and does not retract normally. When it is removed from the test tube to filter paper it wets a large surface area, and it promptly falls to pieces when it is handled; there is no question as to its fragility. The clot of the normal patient, on the other hand, or of the jaundiced patient without a bleeding tendency (Fig. 1-*A*) is smaller, firmer, and definitely re-

tractile, and it can be manipulated both manually and instrumentally with a considerable degree of force without showing any tendency toward disintegration.

Carr and Foote explain the tendency to hemorrhage by seepage through an ineffective clot, an explanation which is undoubtedly correct but which does not go back far enough. Such clots, Kilduffe[18] believes, are associated with a low fibrinogen content, but that explanation scarcely holds, for Moss has shown that in jaundiced patients the fibrinogen content of the blood is normal or even elevated. We ourselves prefer to explain the clot defect on the basis of Quick's prothrombin deficiency theory. If his concept be correct, that the conversion of prothrombin to thrombin is a mass reaction, then a diminution in prothrombin is necessarily followed by a diminution in the production of thrombin, which in turn is followed by a diminution in the production of fibrin, the essential element in the formation of a firm clot. Neither the bleeding time nor the clotting time is necessarily altered, regard-

Fig. 1.—*A*, Coagulation of blood in normal subject. *B*, Coagulation of blood in jaundiced subject with hemorrhagic tendency. Note the large, bulky clot and the small amount of serum expressed, in contrast to the small clot and the large amount of serum expressed in the normal subject.

less of the inadequacy of the mass reaction, since the conversion of fibrinogen to fibrin is merely decreased and not eliminated.

Ivy, McNealy and their associates comment that the tendency to bleed is more likely to be evident twenty-four to forty-eight hours after operation than at operation. Their idea is that in patients with a hemorrhagic diathesis clotting occurs as rapidly as in normal patients but that the abnormally fragile clots are likely to be dislodged by postoperative vomiting, restlessness and similar factors. We agree with them that frank hemorrhage is more likely to occur after operation than during the operative act, but we would emphasize one consideration they have overlooked, that free oozing at operation is characteristic in such cases. This oozing can easily be explained by the character of the clot, which prevents free bleeding but which, because of its porous character, permits seepage. In other words, the clot plugs the severed vessel but it does not plug it effectively, the deficiency being quite as evident in invisible capillaries as in visible veins and arteries. The capillary type of bleeding, which is both treacherous and serious, needs particular emphasis, for its significance is likely to be overlooked. With this reservation

we are in entire accord with the explanation of postoperative hemorrhage in jaundice advanced by the Northwestern group. It is worth adding that as early as 1914 Goodpasture[19] suggested that early solution of a normally formed clot might explain hemorrhage in hepatic disease, in which fibrinolysis tends to be excessive.

Whatever be the correctness of a clot defect as the etiologic factor in the hemorrhagic diathesis in jaundice, our own observations have convinced us that a careful study of the character of the clot can furnish valuable data on which to base a prediction as to whether or not a particular jaundiced patient is likely to bleed. If this be true, and we feel justified in asserting that it is, then it is a worthwhile observation, for so far all of the tests which have been devised to determine this point are either entirely inaccurate or altogether impractical for the surgeon who does not have at his command a well-equipped laboratory and an unusually expert technician.

It is generally agreed that the total and the differential blood counts furnish no information at all. The coagulation time, the bleeding time and the platelet count are likely to be within normal range. The sedimentation time is influenced by too many other factors to be of any special value. The degree of the jaundice, as expressed by the icteric index, and its duration bear no constant relation to the hemorrhagic diathesis. Our observations parallel those of other observers on this point. With an index of 300 and more we have noted not the slightest tendency to hemorrhage, and we have seen fatal bleeding associated with jaundice that was slight or even latent. Carr and Foote, in a group of 14 cases, used all of these tests in every case, as well as the more delicate tests for diffusible and total calcium and for fibrinogen and fibrin, and arrived at the conclusion of the investigators who used these tests separately, that not a single one of them offers any reliable information as to what is going to happen in any given case. The pessimistic opinion of Ivy and his associates seems entirely justified, that the only way at present to determine a bleeding tendency is to wait and see whether the patient bleeds.

Ivy and his coworkers have devised a test in which the factor of capillary retraction is eliminated and the burden of controlling hemorrhage is put squarely upon the factor of coagulation. By the application of the cuff of a sphygmomanometer around the arm, with a pressure of about 40 mm. of mercury, the venous return is cut off and a condition is produced comparable to the clinical state produced by anesthesia, shock, operative trauma, and similar surgical conditions which may result in capillary paresis and hemorrhage. They believe that the determination of the venous pressure bleeding time under these circumstances is a truer index of the ability of the blood to coagulate than is its determination when the factor of capillary retraction is present also. In an extensive series of cases, Ivy found that jaundiced patients with a bleeding tendency showed a definitely prolonged venous pressure bleeding time, while patients without such a tendency showed a bleeding time within normal limits. The physiologic mechanism underlying the test is the theoretical fragility of the clot in hepatic insufficiencies. The bleeding time is frequently normal in these cases when the factor of capillary retraction is

present, but the clots yield to less pressure than do normally formed clots, and there is revealed a latent failure of the smaller blood vessels to contract and retract normally.

Our personal experience with this test is limited and is, unfortunately, confined to cases in which the clot was normal and the patient, by the test we shall describe shortly, showed no evidence of a bleeding tendency. Our theoretical comment is that the clot produced by puncture is so minute that it is unlikely to be seriously affected by the limitation of the venous return, while the arbitrary upper limit of normal coagulation time selected by the authors of the test (four minutes) probably permits the inclusion within it of patients with a bleeding tendency. We have also noted that age is a consideration not to be overlooked when this test is employed. In a series of young normal controls the average venous pressure bleeding time was one minute; the time increased with increasing age, and our oldest control, a woman of seventy years of age, had a bleeding time of four minutes. We shall continue to use the test as an adjunct or confirmatory measure, for its simplicity has much to commend it.

In 1931 Lewisohn reported a series of 7 cases in which he had studied the prothrombin, the fibrinogen, and the antithrombin and had arrived at an index by dividing by antithrombin prothrombin multiplied by fibrinogen. An index above 0.7 he considered furnished a good prognosis, while an index below this figure indicated a tendency to hemorrhage. In the small number of cases which he reported the test seemed accurate, but it is clearly too elaborate to be considered for general use.

Nygaard of the Mayo Clinic, working along somewhat similar lines, reported a far simpler test the following year. He found that the coagulation time of the plasma in 238 normal subjects ranged from 120 to 285 seconds, with a mean of 190 seconds, and that the coagulation time of the plasma in 90 jaundiced subjects ranged from 145 to 490 seconds, with a mean of 250 seconds. While the coagulation time of the plasma in any given case can be evaluated on the basis of the actual figure, he prefers to express it as an index, which is arrived at by dividing the normal coagulation time by the coagulation time of the blood plasma of the patient under consideration. The normal index is 1. In the group Nygaard studied the 6 patients who died of hemorrhage all had indices under 0.75, while all who exhibited a hemorrhagic tendency had indices under 1.1. One criticism of the test is immediately obvious: If 1 is the standard of normal, it seems curious that patients with indices even higher than normal should exhibit a bleeding tendency. The question is properly raised as to whether the plasma clotting time parallels the blood coagulation time. The test, unfortunately, came to our attention too recently for us to use it in the group of patients we are now reporting, but we propose to employ it in the future, for it has the virtue of being far less complicated than many other tests suggested for this purpose.

The Quick test for the determination of prothrombin is apparently a delicate and reliable index of the bleeding tendency in jaundice, but it has the very serious defect of being entirely impractical for general use. In spite of our conviction of its value we have not attempted to employ it, and for surgeons without access to laboratories it cannot even be considered.

The serum volume test of the bleeding tendency in jaundice, which we are presenting herewith, was devised entirely accidentally, during a study of the Grote test[20] for sulphur compounds (the sulphur amino acids, cystine, cysteine and similar substances). This study itself was undertaken almost accidentally, because of our casual recollection of a probably equally casual remark by one of our instructors in the course of our undergraduate medical lectures, to the effect that perhaps the bleeding tendency in jaundice might be explained by the presence of sulphur compounds in the blood. The Grote test seemed to us best fitted for our purposes, and while we were working with it, Carr and Foote published their paper on progressive obstructive jaundice and we found that they also had employed it. So far as we know, no other report of this test has been made.

In the Grote test the presence of sulphur compounds is manifested by the formation of variously colored rings in a definite sequence; the C-S-H group is manifested by a purple red ring, the C-S group by an intense green or blue ring, and the C-S-S-C group by a purple red ring which appears after the addition of potassium cyanide. We were unable to duplicate these color reactions. In some clinical cases, however, sometimes without any intermediary step, we obtained a green ring after the addition of potassium cyanide. What substance is responsible for this green ring we have not been able to determine, nor could the author of the test[21] suggest anything to us regarding it.

Carr and Foote obtained Grote's color rings in their experimental work, while in our own animal work we obtained the green ring almost consistently. The explanation of the difference in the results may perhaps be that Carr and Foote used the test when their animals were in the terminal stage; they do not anywhere state this specifically, but one of their illustrations suggests it. They believe that the clot formed in cases with a hemorrhagic diathesis is influenced by the collection of intermediate products of protein metabolism, such as cysteine and related forms of mercaptan, the result, as they point out, being a nonretractile, friable jelly instead of a strong occluding mesh.

It had been our hope, when we first secured the green ring instead of the usual Grote color rings, that perhaps we had unwittingly stumbled upon a test which might indicate a tendency to bleeding in jaundice. We soon found that whatever else this reaction might indicate, it furnished no reliable information on that point. During the course of these studies, however, we did observe, again merely by accident, that a certain patient with obstructive jaundice, who showed clinically many purpuric spots and hemorrhages from the mucous membrane, had a peculiarly large and bulky clot, from which a notably small amount of serum was expressed for use in the Grote test (Fig. 1-*B*). Thereafter we began to study the serum volume in all such cases and we soon found that we had a test which we have been increasingly sure is a reliable indication of the bleeding tendency in jaundice.

The test is exceedingly simple. An arbitrary amount of blood, preferably 3 c.c., is collected in a graduated tube and allowed to stand at room temperature for four hours, at the end of which time the clot is removed and

studied for firmness, retractility and similar characteristics. The serum volume is then read and the index calculated.

In a control group of 25 nonjaundiced and apparently normal male subjects, with blood counts, platelet counts, coagulation time, and bleeding time within normal limits, we found that at four hours the serum volume was equivalent to 50 per cent of the blood volume. This same ratio was maintained in some of our jaundiced patients, while in others, notably the patient who furnished us with the clue and who proved the ease, as it were, by her frank purpura, the serum volume was decidedly below this level. We therefore developed a serum volume index, which equals the serum volume of the patient studied over half the volume of the blood withdrawn for the study. We consider 1 as the standard of normal and consider indices below this normal as progressively indicative of a tendency to hemorrhage. The retractility of the clot, in our experience, is always related to the level of the index, and serves as an additional check.

Only one precaution is necessary, a routine blood count to demonstrate a possible anemia. If anemia be present, it is obvious that the serum volume will be greater than half the blood volume and the proper correction must be made when the index is calculated. Otherwise the test is performed exactly as we have described it, and its simplicity, we feel, makes it a practical method for any physician to use anywhere. Nothing is needed but a syringe and a graduated test tube, plus the naked eye.

As Table I shows, there is no correlation between the routine blood studies, the icteric index, the Grote test, and this serum volume test. Also, though this is not shown in the table, there is no constant correlation between

TABLE I

OBSTRUCTIVE JAUNDICE

CASE	R.B.C. COUNT	PLATELET COUNT	ICTERIC INDEX	COAG. TIME	GROTE TEST	SERUM VOL. INDEX
1	4,770,000	371,600	125.0	5.0	Green	0.93
2	4,170,000	287,730	66.0	6.75	Green	----
3	--------	------	167.0	--	Purple	----
4	4,360,000	283,000	66.6	3.0	Green	0.53
5	4,660,000	344,840	26.0	2.0	Neg.	0.80
6	3,680,000	249,240	34.4	5.0	Neg.	0.89
7	4,410,000	251,270	52.6	5.5	Neg.	0.66
8	3,570,000	217,770	200.0	3.0	Neg.	1.00
9	3,160,000	420,000	71.5	4.5	Green	1.00
10	4,790,000	531,690	125.0	3.5	Neg.	0.83
11	4,020,000	241,200	250.0	8.5	Green	0.66
12	4,860,000	529,740	62.0	6.0	Neg.	0.30
12A	4,290,000	364,650	100.0	8.0	Green	0.30*
13	5,840,000	502,240	333.0	7.0	Green	0.48
14	4,290,000	351,780	56.0	7.0	Neg.	0.10
15	--------	------	167.0	--	Green	----
16	3,610,000	202,160	67.0	5.5	Green	0.93
17	4,180,000	175,500	100.0	5.0	Green	1.0
18	4,370,000	413,330	43.0	5.0	Neg.	0.66
19	3,670,000	433,060	110.0	5.0	Green	0.86
20	4,750,000	250,000	50.0	4.5	----	1.0
21	5,060,000	330,000	200.0	6.0	----	1.0
22	4,680,000	220,000	200.0	7.0	----	1.0

*12 and 12A are the report of separate tests (at an interval of ten days) on the same patient. Note that the serum volume index remains the same, in spite of the alteration in the icteric index, coagulation time and Grote test.

the duration of the jaundice and the serum volume index. Case 21 proves this point very clearly. This patient, whose common duct had been damaged at cholecystectomy seven months previously, had developed a biliary fistula, which at intervals became obstructed; during these periods she was deeply jaundiced, and at no examination during the whole time was bile detected in the stools. When the serum volume test was run, the day before closure of the fistula was attempted, the patient was very ill; the icteric index was 50 and the jaundice for some days had been of the bronze type. The serum volume test was normal, the clot was small, firm and of normal retractility, and the Quick hippuric acid liver function test was also normal. In spite of her surgeon's forebodings her convalescence was without incidence, and there was no evidence of oozing or frank hemorrhage at any time.

In Case 4 the serum volume test was 0.53 immediately after admission, but after treatment was elevated to approximately normal (0.86) and both operation and convalescence were without incident. Case 13 is particularly worthy of comment. This man's jaundice was of brief duration, two weeks, but his icteric index on admission was 333. All his other tests were normal, except the hippuric acid liver function test of Quick, which was 1.859, slightly over 60 per cent of normal. With proper preparation the serum volume index rose from 0.48 on admission to normal (1), and neither at operation nor at postmortem was there the slightest trace of hemorrhage or oozing. He died a typical hyperpyrexia liver death, as the markedly decreased liver function had led us to fear. Such a combination of circumstances might perhaps be assumed to prove that the coagulability of the blood in jaundice bears no relation to the degree of liver damage. That may be true, but the assumption is also feasible that perhaps, had the patient lived longer (he died within fifty-four hours of operation), the tendency to hemorrhage might have reappeared as the damaged liver progressively failed in its function and released more and more toxins into the circulation. Such theories are purely speculative and should be so regarded at this time.

All of these patients were prepared for operation by the standard measures, including glucose intravenously. Transfusion was employed in Cases 4 and 13. Neither at operation nor thereafter did any of the patients show

TABLE II

NONOBSTRUCTIVE JAUNDICE

CASE	R.B.C. COUNT	PLATELET COUNT	ICTERIC INDEX	COAGULATION TIME	GROTE TEST	SERUM VOL. INDEX
1	5,260,000	520,740	100.0	4.5	Green	0.80
2	5,430,000	282,360	125.0	8.5	Green	1.00
3	3,440,000	209,840	84.0	5.0	Neg.	----
4	4,790,000	225,130	77.0	7.0	Neg.	----
5	--------	------	84.0	--	Green	----
6	4,630,000	245,390	62.5	5.0	Green	0.73
7	3,100,000	148,800	17.2	5.0	Neg.	1.0
8	5,040,000	317,520	166.0	6.5	Green	----
9	4,140,000	252,540	125.0	8.0	Neg.	----
10	3,080,000	224,840	250.0	8.0	Green	1.00
11	--------	------	67.0	--	Neg.	----
12	5,580,000	345,960	28.5	4.0	Green	1.00
13	4,140,000	400,000	98.0	2.0	Green	1.00

any tendency toward hemorrhage. It is regrettable that double preoperative tests were made in only two cases, but the full significance of these studies was not realized at the time.

We have also used this serum volume test on 13 nonsurgical jaundiced patients (Table II). We have, of course, no way of proving what would have happened to them had operation been necessary, but we see no reason to doubt the correctness of the test in these subjects.

We suggest the use of the serum volume test postoperatively, after choledochostomy, to determine how long drainage may safely be continued. Often in such cases prolonged drainage is indicated for one reason or another, and yet such patients, it is well known, under such circumstances sometimes develop a tendency to hemorrhage which was not present preoperatively. The serum volume test will furnish a reliable index of this new tendency, we believe, though we have as yet had no opportunity to put our belief to the test.

The rationale of the preoperative preparation of jaundiced patients needs only brief discussion. The liver function is sometimes surprisingly good, even in apparently poor risk patients, as the case (No. 21) which we have just discussed clearly shows. But usually there is more or less damage to be combated. This aspect of biliary disease we shall discuss in detail in a communication, now in course of preparation, in which we report our results with the Quick hippuric acid liver function test. To combat this damage it has long been accepted that the proper treatment is a high carbohydrate diet, with glucose orally or intravenously as indicated. To this therapy should be added the administration of calcium, as suggested experimentally by Wright[22] and by Lee and Vincent[23] and as advocated clinically by Waltman Walters. The theory of calcium deficiency has long since been disproved, but the excellent results of this type of therapy cannot be controverted, and there is no doubt, whatever the mechanism, that it has a salutary effort upon the coagulation time. Perhaps, as Ravdin's[24] studies would seem to indicate, it acts by way of the liver. Whatever be the basis, rational or irrational, the results warrant the use of calcium.

The value of transfusion under these circumstances is also well established. Quick believes, and we are inclined to agree with him, that the beneficial effects of even small transfusions are to be explained on the basis of a replacement of prothrombin. He has shown that there is a wide margin of safety in prothrombin concentration, and it may well be that even its partial restoration will sufficiently improve the plasma clotting time to bring it within normal range. Walters, by hourly observations, has shown that there is a period of eight to ten hours after the use of transfusion and other coagulant agents during which the coagulation time is normal, and he suggests that this is the optimum chance for operation on those patients in whom earlier observations have shown this improvement is not maintained. We grant the force of his argument so far as operation is concerned, though it does not take care of the risk of postoperative hemorrhage, which, as we have already mentioned, is rather greater than the risk of hemorrhage at operation.

Hawkins'[25] experimental work, supplemented by Judd's observations, introduces another interesting possibility. Hawkins has shown that the blood

coagulation time is accelerated by the breathing of oxygen, which would seem to indicate a direct relationship between the oxygen saturation curve of the blood and the coagulation time. Judd notes that the oxygen saturation of the blood is decreased in patients with hepatic disease, and suggests oxygen inhalations as a corrective measure. His conclusion that perhaps one result of transfusion is to improve the oxygen saturation curve seems entirely rational.

The number of cases we are reporting is small, but we are putting them on record in order that the test we are proposing may be used by others, particularly by those surgeons and research students who have interested themselves in the problem of the hemorrhagic diathesis in jaundice. In partial explanation of our small number of cases we might comment upon a point which we have noted at some length elsewhere, that the incidence of hemorrhage in jaundice and the mortality from it in this community are apparently considerably less than seems to be the case elsewhere. In a recent study[26] of 100 consecutive deaths after biliary surgery in Charity Hospital in New Orleans, we found only 4 patients in whom hemorrhage could be considered a cause of death. The same low incidence is commented upon by Rives, Romano and Sandifer[27] in an analysis of 96 cases of carcinoma of the pancreas. In this group only 5 jaundiced patients presented any sort of bleeding as a symptom and only 4 jaundiced patients died of postoperative hemorrhage. These figures in no wise approximate those of Walters and other observers, and we can offer no explanation for them.

SUMMARY

1. The problem of the hemorrhagic diathesis in jaundice is still unsolved.

2. Only the prothrombin deficiency theory, the liver damage theory and the defective clot theory are now tenable. These theories are briefly reviewed.

3. The defective clot theory is examined in the light of our personal observations, which corroborate the observations of others, and an explanation for it is advanced.

4. The tests now in use for predicting the hemorrhagic tendency in jaundice are reviewed, and it is pointed out that they are generally either unreliable or impractical.

5. The serum volume test is described and is advocated as a simple and reliable test of the bleeding tendency. Illustrative cases are adduced.

6. The preoperative preparation of jaundiced patients is discussed.

7. The low incidence of hemorrhage in jaundice in this community is commented upon.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TAKATA-ARA TEST, In Hepatic Diseases, Cuttle, T. D., Kerr, E. G., and Wolff, W. A. Bull. Ayer Clin. Lab., Penna. Hosp. **3**: 99, 1937.

One hundred and thirty-six Takata-Ara tests on 76 patients studied with special reference to the presence of hepatic disease show that this test has a limited diagnostic and prognostic value. It is not a specific test for cirrhosis of the liver, bears no relation to the presence or absence of jaundice, and may be positive in conditions without demonstrable hepatic damage. A positive test depends largely but not entirely upon an abnormally increased globulin content of the fluid examined.

OVARIAN TUMORS, Cytology of, Page, R. C., and MacCarty, W. C. Arch. Path. **24**: 1, 1937.

Studies of nuclei and nucleoli were made on typical cells of a group of 33 malignant and 49 nonmalignant tumors of the ovary. By determining the actual areas of the nuclei and nucleoli, it was found that the ratio of the area of the nucleus of the malignant cell to that of the nonmalignant cell was 1.35:1. The ratio of the area of the nucleolus of the malignant cell to that of the nonmalignant cell was 3.9:1.

The nuclear-nucleolar ratio of the malignant cell was found to correspond to the grade of malignaney in that as the grade of malignancy increases, the nuclear-nucleolar ratio becomes smaller. The nuclear-nucleolar ratio of malignant tumors of the ovary is less than a third that of nonmalignant tumors.

This study again emphasizes the value of a knowledge of the cytology of malignant and nonmalignant tissues, which enables one to make a diagnosis in the early stages of cancer.

TUMORS: Hypernephroid of Kidney, Whitmore, E. R. Southern M. J. **29**: 1051, 1936.

It is suggested that, along the lines of the general tendency of the study of neoplastic diseases today, the intensive and somewhat academic discussion of the origin of certain tumors of the kidney may well give way to more practical considerations of diagnosis and treatment, leaving to newer methods of investigation the solution of the problem of the origin of these tumors.

Meanwhile, one may well accept Lubarsch's suggestion that these tumors be called by a name that would prejudice as little as possible. He suggested: "hypernephroid new growths, or still better, tumors of the type of the adrenal with or without destructive character."

With improved methods of diagnosis and treatment it becomes doubly important that we increase our knowledge of the variation in nature or type of these tumors and concentrate on diagnosis and treatment of them.

Three cases are cited to indicate the varying nature or type of these tumors. In one case, the clinical picture was that of an acute kidney condition. At operation, there was a ruptured hemorrhagic cyst of the kidney. The kidney was removed and on microscopic examination groups and strands of clear tumor cells were found in the thick wall of the cyst. The patient is well three and one-half years after operation.

In another case, there is the usual clinical picture of these tumors, in which metastasis had occurred before operation and caused the death of the patient one year later.

In a third case, pulmonary symptoms dominated the clinical picture. At necropsy there were a tumor in the left kidney, nodules in both kidneys, and extensive metastases in the lymph nodes and lungs.

With improved diagnosis, especially with the added aid of x-rays, we can look forward to better therapeutic results from surgery, and radiation therapy, either singly or combined. The demonstration that between 90 and 95 per cent of these tumors are radiosensitive, and that the necessary therapeutic dose of radiation does not injure the normal renal tissue, makes preoperative irradiation a very important adjunct to surgery in their treatment.

TYPHUS FEVER, Laboratory Methods for Recognition of, Report of Referee Suppl. to, Am. J. Pub. Health **27**: 141, 1937.

SEROLOGIC PROCEDURES

Examination of Serum for Agglutinins of Proteus X.

I. *Outfit.*—A sterile glass container having a capacity of 10 to 15 ml. This container should be tightly stoppered and in a mailing case conforming to the postal laws.

II. *Specimen.*—At least 5 ml. of blood should be collected and placed in the container without oxalate, citrate or preservative of any kind.

III. *Time of Taking Specimen.*—A series of 3 blood samples should be tested in both questionable and clinically positive cases. The first taken as soon as the nature of the infection is suspected or when the rash appears, the second between the tenth and fifteenth days, and the third during early convalescence. (A marked increase in titer established by repeated examination gives an absolutely conclusive diagnosis.)

IV. *Preparation of the Specimen.*—The serum is separated from the clot by centrifugation in the usual manner. (The significance of reactions obtained with badly hemolyzed serum is questionable.)

V. *Antigen Preparation.*—Fresh suspensions of living bacteria which give the highest degree of sensitiveness and accuracy should be used.

A. *Strains of Proteus:* Proteus OX19, Proteus OX2, Proteus HXK, and Proteus OXK should be used when necessary. In most instances Proteus OX19 should be sufficient.

B. *Maintenance of Cultures:* 1. All strains with the exception of Proteus HXK should be retained in a nonmotile state. To insure working with a nonmotile culture checks should be made at least once a week.

2. The Proteus strains should be carried on either nutrient agar slants or on agar slants prepared from fresh meat bouillon. The slants should be dehydrated sufficiently to remove most of the water of condensation which increases the possibility of the development of motile variants. Excess amounts of water of condensation tend also to inhibit the growth of certain Proteus X strains.

3. The reaction of all media should be pH 7.4.

4. The morphologic, biochemic, and serologic reactions of the diagnostic strains should be checked at intervals of 6 months, since changes in these characteristics indicate instability of the organism.

5. The diagnostic strains should be checked at least once each quarter to exclude spontaneously occurring variants. Direct agglutination at 37° C. with 5 known antisera, i.e., *Eberthella typhosa, Salmonella Schottmulleri, Salmonella paratyphi, Shigella paradysenteriae* Army and Flexner should be sufficient.

C. *Antigen Suspension:* The antigen suspension should be prepared in 0.85 per cent salt solution from an eighteen- to twenty-four-hour culture of living organisms. All suspensions should be filtered once through a single fluted filter paper. The turbidity of the suspension on the Gates apparatus should read 8.5 to 9.5.

Note: More uniform turbidities may be obtained when the same technician standardizes the daily suspensions. Other turbidity standards which give comparable densities and consistently uniform results may be used.

VI. *Technic of Test.*—

A. The test is set up in agglutination tubes (75 × 10 mm.) by mixing thoroughly 0.5 ml. of serum dilutions and 0.5 ml. of antigen suspension. Serum dilutions of 1:10 through 1:320 (final dilutions 1:20 through 1:640) are usually sufficient.

Both salt solution and positive serum controls should be made each day tests are done. Tests and controls are incubated two hours at 37° C. followed by overnight in the refrigerator at 8° C.-10° C.

VII. *Tabulation of Results.*—The degree of agglutination is recorded as follows:

++++ = Complete agglutination, clear supernatant fluid (100 per cent)
+++ = Agglutination nearly complete, supernatant fluid slightly opalescent (75 per cent)
++ = Definite agglutination, supernatant fluid opalescent (50 per cent)
+ = Agglutination discernible, supernatant fluid cloudy (25 per cent)
± = Very fine agglutination barely discernible (questionable reaction)
0 = No agglutination, suspensions correspond to salt solution control.

VIII. Report.—Results should be reported in actual titers obtained. A titer of 1:100 should be reported as positive when properly controlled cultures of the 0 Type are used in the test. It is always advisable to obtain more than one specimen from a patient, as the subsequent rise in titer during infection and fall during convalescence is definitely diagnostic.

BLOOD: The Macropolycyte in Health and Disease in Iraq, Kennedy, W. P., and MacKay, I. J. Path. & Bact. **44**: 701, 1937.

The term macropolycyte was first suggested by Sir Humphry Rolleston to describe a giant type of polymorphonuclear leucocyte which exceeds 16 in diameter and may be over 25. The normal polymorph in stained films averages 10 to 12 in diameter, occasionally reaching 14.

Macropolycytes were found in 1.3 per cent of 690 normal individuals and in 4.76 per cent of 609 pathological individuals in Iraq. The cells are described and the observations recorded are advanced as further support of the hypothesis that environmental factors incidental to life in Iraq result in an alteration of the hemopoietic balance.

LEUKOPENIC INDEX: Critical Study of Leukocyte Behavior During Gastric Analysis, Long, C. F. J. A. M. A. **109**: 23, 1937.

With the technic of Vaughan and the criteria of Rinkel, a study was made of 100 unselected patients from a gastrointestinal clinic. By means of leucocyte counts during gastric analysis the authors attempted to survey any possible constant relationship existing between the acid values and leucocyte behavior. None was found.

In this series were found fourteen patients with proved peptic ulcer. In these, too, there was no constant relationship between acid values and the leucocyte counts.

RENAL FUNCTION: Excretion of Ferrocyanid in Man in Relation to Urea Clearance, Gordon, W. Am. J. M. Sc. **192**: 208, 1936.

The ferrocyanid excretion test of renal function was compared with the simultaneous urea clearance in 102 subjects with a range of clearance levels from 6 to 130 per cent. Following intravenous injection of 0.28 gm. of sodium ferrocyanid, normal subjects excreted at least 24 per cent in one hour, 35 per cent in two hours, and 50 per cent in three hours. There was a correlation of about 0.7 between urea clearance and ferrocyanid excretion for each of these three periods. One hour proved to be an adequate length of time for the test. The administration of ferrocyanid did not alter the clearance. In those instances in which the tests gave discordant results other evidence pointed to error in each of the two methods with about equal frequency. Considerable value in detection of impairment of renal function may be expected of the ferrocyanid test, but its value in observing the progress of renal disease cannot be judged yet and is somewhat more uncertain. The test is simple and harmless in

most instances. Great care is required in estimating ferrocyanid in the presence of hematuria and some accuracy is sacrificed if much blood is present in the urine. Patients with prostatic hypertrophy had dysuria and inability to void in some instances.

CREATININE, Colorimetric Determination of, in Urine and Blood With 3.5 Dinitrobenzoic Acid, Bolliger, A. Med. J. Australia 2: 818, 1936.

For this colorimetric determination the following reagents are necessary.

1. A 10 per cent solution (approximately) of 3.5 dinitrosodiumbenzoate. This is prepared by adding slowly under constant stirring 20 c.c. of 0.5 N sodium hydroxide to 2.2 gm. of 3.5 dinitrobenzoic acid. Most of the dinitrobenzoic acid goes into solution, which is filtered when it has become practically colorless.

In order to get a satisfactory solution, the 3.5 dinitrobenzoic acid used has to be of high purity. It is best prepared by nitration of chemically pure benzoic acid, according to the method of Shukoff. The heating on the sand bath has to be stopped as soon as the nitrous fumes are driven off. Then the dinitrobenzoic acid formed is allowed to crystallize out from the sulphuric acid solution. The dinitrobenzoic acid is separated from the mother liquor by filtration. A funnel containing a fritted glass plate is most convenient for this operation. Then the crystals are transferred into about ten volumes of water and filtered again. Fifty grams of benzoic acid yield about 45 gm. of dinitrobenzoic acid. This product now has to be recrystallized from glacial acetic acid at least three times. The test for satisfactory purity is as follows: Add to 2.0 c.c. of water 0.8 c.c. of the dinitrosodium benzoate solution and 0.4 c.c. of normal sodium hydroxide. The mixture should remain practically colorless. If a brown tinge appears, the dinitrobenzoic acid has to be further purified.

2. Normal sodium hydroxide.

3. Stock standard 0.1 per cent solution of creatinine. Dissolve one gram of creatinine in one liter of 0.1 normal hydrochloric acid.

4. Dilute standard creatinine solutions are prepared by diluting with water after neutralizing the hydrochloric acid present in the stock solution. These dilute standards of neutral reaction do not keep and have to be made fresh daily.

Transfer 2 c.c. of the fluid to be examined, which preferably should be colorless and of neutral reaction, to a test tube and add 0.4 c.c. of a 10 per cent solution of 3.5 dinitrosodium benzoate and 0.4 c.c. of normal sodium hydroxide. In another test tube treat 2.0 c.c. of the standard solution in the same manner. After letting the two test tubes stand in the dark for ten minutes compare them in a colorimeter. If the creatinine is present in a concentration of more than 5 mg. per cent, immediately after the sodium hydroxide is added a purple color will appear which deepens considerably after further standing. After about fifteen minutes it will begin to change toward red, and after about thirty minutes it will begin to fade. Ultimately it will become yellow. With smaller concentrations a more reddish color will be obtained, which may take a few minutes to appear. In this case, wait fifteen minutes before reading.

It is essential that the depth of color in standard and unknown differs by not more than about 50 per cent. To insure this it is well to prepare more than one standard with each test.

By this test the presence of 0.2 mg. of creatinine may still be detected and estimated.

DETERMINATION OF CREATININE IN URINE

The reaction of 3.5 dinitrobenzoic acid with creatinine is readily applied to the determination of creatinine in urine. However, one has to bear in mind that best results are obtained with creatinine concentrations of from two to 15 mg. per cent. Therefore, concentrated and highly colored urine has to be diluted from ten to twenty times to obtain results within the range mentioned. With this dilution it is usually possible to avoid interference due to the color of the urine. Very dilute and usually correspondingly pale urine with low creatinine content should be diluted only about five times in order to obtain a sufficiently strong color.

LYMPHATIC SYSTEM, The Blood Pictures in the Primary Diseases of, Wiseman, B. K. J. A. M. A. **107**: 2016, 1936.

The primary diseases of the lymphatic system that may be favorably influenced by irradiation consist of lymphosarcoma, lymphatic leucemia and Hodgkin's disease. It is probable that, excepting the benign hyperplasias, all primary diseases of the lymphoid tissues difficult to classify belong in one of these three classes.

All grades of lymphatic leucemia may be encountered, ranging from the pathologic state without leucemia, through benign states resembling chronic lymphocytosis, to those which are very rapidly progressive and rapidly fatal. Many of these types show very little resemblance, cytologically, clinically or histologically, to neoplasia.

Lymphosarcoma may be characterized hematologically either by a leucemic or by a nonleucemic blood picture. The former constitutes a type of leucemia (leucosarcoma) which possesses special features of neoplasia and is rapidly progressive and very radiosensitive.

Hodgkin's disease, usually but not always, possesses a characteristic blood picture. These characteristics are interpreted as suggesting an alteration of the reticulum cell-monocyte maturation cycle arising from an infectious agent.

Careful blood studies in the control of radiation therapy in this group of diseases are necessary if optimum therapeutic results are to be obtained.

ARTHRITIS, Rheumatoid, Comparative Evaluation of Commonly Employed Diagnostic Tests, Short, C. L., Dienes, L., and Bauer, W. J. A. M. A. **108**: 2088, 1937.

On a group of 49 patients with active rheumatoid arthritis, 4 commonly employed "measuring sticks" were evaluated.

The sedimentation rate was found positive in 92.2 per cent of the tests, with the Schilling count only slightly less accurate (87 per cent). The Vernes test and streptococcus agglutination reactions were positive in approximately 50 per cent (58.3 and 53.1, respectively).

In cases of a year's duration or under, the sedimentation rate and the Schilling count were positive less often than in cases of longer duration. The other two tests showed approximately similar results in each group.

The sedimentation rate was slightly more accurate in severe than in mild cases. The Vernes test was markedly so, while the other two showed little difference.

The authors' results agree in the main with those reported in the literature.

From the standpoint of practicability and accuracy, the sedimentation rate is the most useful laboratory test thus far in common use to aid in the recognition and evaluation of patients with active rheumatoid arthritis.

NEOPLASMS: Nuclear Sizes in Growth Disturbances, With Special Reference to the Tumor Cell Nucleus, Ehrich, W. E. Am. J. M. Sc. **192**: 772, 1936.

The present report is based upon the measures of 220,000 nuclei of 230 specimens, half of which were growth disturbances.

In all tissues studied, normal as well as pathologic, several nuclear classes were found the sizes of which were in the proportions of Heidenhain's law of growth.

The nuclear sizes of normal tissues are by no means constant; they vary not only in the same organ in different individuals, but also in different organs of the same individual. In physiologic variations apparently functional influences play the main rôle.

In hypertrophy and atrophy several mechanisms are involved. In certain organs we find an increase or decrease in the size of the nuclei in the proportions of Heidenhain's law with a shift from the number of the smaller to the larger cells or vice versa (liver, prostate). In other organs we merely observe an increase or decrease in the number of cells with or without change of their size (lymphatic and lymphoid tissue). In still others we mainly find a simple swelling or shrinkage outside of the proportions of Heidenhain's law (breast, thyroid). Which of these avenues is chosen depends largely upon the regeneratory ability of the tissues and probably upon the ability of functional swelling or shrinkage of their cells.

Benign tumors are characterized by cells larger than their mother cells. The enlargement is due to simple swelling and not to true growth.

Malignant tumors are characterized by cells which are double or fourfold the size of their mother cells, and which probably arise by derailing from the normal track of growth during cell division. The malignant tumor cell is not of embryologic cell, but a special cell that occurs in malignant tumors only. The double or quadruple size of these cells is an anatomical expression of the anaplasia or kataplasia of malignant tumors so long searched for.

BLOOD GROUPING, In Forensic Medicine, Gettler, A. O., and Kramer, H. E. J. Immunol. **31**: 321, 1936.

Fresh blood samples of known group were smeared on cloth, paper and metal implements, and allowed to stand for various intervals of time. The dried blood stains were then again tested. When putrefaction of the blood samples set in before they dried, as many as 14 out of 18 wrong groups were obtained. It is of importance to note that not only were agglutinogen or agglutinin destroyed during the drying or ageing of the blood stains, but in some instances changes occurred which yielded reactions simulating those of some particular agglutinogen.

The authors strongly advise against the use of blood grouping of partially decomposed (and contaminated) blood stains, since their experiments definitely prove that 100 per cent correct results cannot be obtained.

ALBUMINURIA, Orthostatic, The Renal Lesion in, Rytand, D. A. Arch. Int. Med. **59**: 848, 1937.

Five subjects with orthostatic albuminuria who had been studied roentgenographically after the injection of diodrast were studied with regard to the Addis urea ratio, the Rehberg glomerular filtration rate and the Addis count of the urinary sediment.

In the two subjects in whom unilateral changes related to posture were revealed roentgenographically, the Addis urea ratio and the glomerular filtration rate fell in the second hour with the subject erect to approximately one-half the original level with the subject recumbent. In one of these subjects, who had unusually severe albuminuria, the results of the function test were diminished even when he was supine. In the two subjects with gross anomalies, posture did not affect the renal function. In the fifth case there was a 20 per cent fall in the renal function when the subject stood.

In these cases, in addition to proteinuria, standing erect results in the appearance of large numbers of renal epithelial cells and broad granular and hyaline casts in the urinary sediment, a small increase in the red blood cell count occurs inconstantly and congo red escapes into the urine. In brief, there is a transitory renal degenerative lesion.

Item

Mississippi Valley Medical Society

The Mississippi Valley Medical Society offers a cash prize of $100.00, a gold medal and a certificate of award for the best unpublished essay on a subject of interest and practical value to the general practitioner of medicine. Entrants must be ethical licensed physicians, residents of the United States and graduates of approved medical schools. The winner will be invited to present his contribution before the next annual meeting of the Mississippi Valley Medical Society (September 28, 29, and 30, 1938), the Society reserving the exclusive right to first publish the essay in its official publication, the Radiologic Review and Mississippi Valley Medical Journal. All contributions shall not exceed 5,000 words, be typewritten in English in manuscript form, submitted in five copies, and must be received not later than May 15, 1938. Further details may be secured from the Secretary, Harold Swanberg, Mississippi Valley Medical Society, 209-224 W. C. U. Building, Quincy, Illinois.